08463107500V5.0

# A1CD

**Hemolyzing reagent**

## Order information

| REF | ℹ️ | CONTENT | | Analyzer(s) on which **cobas c** pack(s) can be used |
|---|---|---|---|---|
| 08463107190 | 08463107500 | Hemolyzing reagent (50 mL) | System-ID 2069 001 | **cobas c** 303, **cobas c** 503 |

**English**

### System information
**A1CD:** ACN 20690

### Intended use
The hemolyzing reagent is used as diluent for the Tina-quant Hemoglobin A1c assays on **cobas c** systems.

### Summary
The hemolyzing reagent is used as a diluent to hemolyze whole blood samples (whole blood application) and for the dilution of the calibrator (whole blood application and hemolyzate application). During predilution the TTAB[a] in the hemolyzing reagent serves as a detergent to eliminate interference from leukocytes (TTAB does not lyse leukocytes).

A1CD Hemolyzing reagent is concentrated fourfold and is automatically diluted by the analyzer prior to use.

a) Tetradecyltrimethylammonium bromide

### Reagents - working solutions
Aqueous buffered matrix, pH 7.25; TTAB: 36 g/L; phosphate buffer: 80 mmol/L; stabilizer; preservative

The reagent is in position B.

### Precautions and warnings
For in vitro diagnostic use for laboratory professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.
Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Danger

| H317 | May cause an allergic skin reaction. |
|---|---|
| H318 | Causes serious eye damage. |
| H410 | Very toxic to aquatic life with long lasting effects. |

**Prevention:**

| P261 | Avoid breathing mist or vapours. |
|---|---|
| P273 | Avoid release to the environment. |
| P280 | Wear protective gloves/ eye protection/ face protection. |

**Response:**

| P305 + P351 + P338 + P310 | IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor. |
|---|---|
| P333 + P313 | If skin irritation or rash occurs: Get medical advice/attention. |
| P391 | Collect spillage. |

**Hazardous components:**

- tetradonium bromide
- 2-methyl-2H-isothiazol-3-one hydrochloride

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

### Reagent handling
Ready for use

### Storage and stability

| Shelf life at 2-8 °C: | See expiration date on **cobas c** pack label. |
|---|---|
| On-board in use and refrigerated on the analyzer: | 4 weeks |

When storing at temperatures below 3 °C the reagent may become cloudy. This has no effect on the function of the reagent and is reversible at higher temperatures. It is therefore recommended to equilibrate the reagent to room temperature for approximately 10 minutes and mix thoroughly prior to use.

### Materials provided
See "Reagents – working solutions" section for reagents.

### Assay
Use A1CD Hemolyzing reagent as specified in the relevant Method Sheet for the system reagents.

The hemolyzing reagent is used for the predilution of whole blood samples (whole blood application) and for the dilution of the calibrator (whole blood application and hemolyzate application). A1CD Hemolyzing reagent must be available on the analyzer otherwise the calibration cannot be performed. The exact volumes are programmed in the respective test settings on the system.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

### Symbols
Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

| CONTENT | Contents of kit |
|---|---|
| → | Volume for reconstitution |
| GTIN | Global Trade Item Number |
| Rx only | For USA: Caution: Federal law restricts this device to sale by or on the order of a physician. |

CE

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⬡ Roche

0108056668500V7.0

# A1CX3

**Tina-quant Hemoglobin A1cDx Gen.3**

**Order information**

| REF | i | CONTENT | | Analyzer(s) on which **cobas c** pack(s) can be used |
|---|---|---|---|---|
| 08056668190 | 08056668500 | Tina-quant Hemoglobin A1cDx Gen.3 (500 tests) | System-ID 2066 001 | **cobas c** 303, **cobas c** 503 |
| 08445699190 | 08056668500 | Tina-quant Hemoglobin A1cDx Gen.3 (200 tests) | System-ID 2066 002 | |

Materials required (but not provided):

| 04528417190 | Calibrator f.a.s. HbA1c (3 × 2 mL) | Code 20674 | |
|---|---|---|---|
| 05479207190 | PreciControl HbA1c norm (4 × 1 mL) | Codes 20002-20003 | |
| 05912504190 | PreciControl HbA1c path (4 × 1 mL) | Codes 20012-20013 | |
| 08463107190 | A1CD (Hemolyzing Reagent) (50 mL) | System-ID 2069 001 | |
| 08463093190 | SCCS (Special Cell Cleaning Solution) (50 mL) | System-ID 2905 001 | |
| 11488457122 | Hemolyzing Reagent for Tina-quant HbA1c (1000 mL) | For Hemolysate Application only | |

## English

## System information

Whole Blood Application - Standardized according to IFCC transferable to DCCT/NGSP

| HBW3: | ACN 20660 | Hemoglobin (Hb) |
|---|---|---|
| A1W3: | ACN 20661 | Hemoglobin A1c (HbA1c) |
| RWD3: | ACN 20662 | Ratio % HbA1c (acc. to DCCT/NGSP) |
| RIW3: | ACN 20667 | Ratio mmol/mol HbA1c (acc. to IFCC) |
| A1CD: | ACN 20690 | Hemolyzing reagent |

Hemolysate Application - Standardized according to IFCC transferable to DCCT/NGSP

| HBH3: | ACN 20663 | Hemoglobin (Hb) |
|---|---|---|
| A1H3: | ACN 20664 | Hemoglobin A1c (HbA1c) |
| RHD3: | ACN 20665 | Ratio % HbA1c (acc. to DCCT/NGSP) |
| RIH3: | ACN 20666 | Ratio mmol/mol HbA1c (acc. to IFCC) |
| A1CD: | ACN 20690 | Hemolyzing reagent |

## Intended use

In vitro test for the quantitative determination of mmol/mol hemoglobin A1c (IFCC) and % hemoglobin A1c (DCCT/NGSP) in whole blood or hemolysate on **cobas c** systems. HbA1c determinations are useful for monitoring of long-term blood glucose control in individuals with diabetes mellitus. Moreover, this test is to be used as an aid in diagnosis of diabetes and identifying patients who may be at risk for developing diabetes.

## Summary

Hemoglobin A1c measurements performed with this assay in whole blood or hemolysate, are useful for monitoring of long-term blood glucose control in individuals with diabetes mellitus. Moreover, this test is to be used as an aid in diagnosis of diabetes and identifying patients who may be at risk for developing diabetes.

Hemoglobin (Hb) is the red-pigmented protein located in the erythrocytes, whose primary function is the transport of oxygen and carbon dioxide in blood. Hb is a globular protein composed of four globin subunits, each containing a heme moiety able to bind one oxygen molecule. Therefore, each Hb molecule can bind up to four oxygen molecules.[1] Hb consists of a variety of subfractions and derivatives, including glycated hemoglobins, formed by the attachment of various sugars to the Hb molecule. The set of glycated hemoglobins includes HbA1 and other non-enzymatically formed hemoglobin-glucose adducts; HbA1 is made up of HbA1a, HbA1b, and HbA1c. HbA1c is the major fraction of glycohemoglobin. It is formed in 2 steps by the non-enzymatic reaction of glucose with the N-terminal amino group of the β-chain of normal adult Hb (HbA). The first step is reversible and yields labile HbA1c. This is rearranged to form stable HbA1c in a second reaction step.[2]

In the erythrocytes, the relative amount of HbA converted to stable HbA1c increases with the average concentration of glucose in the blood. The conversion to stable HbA1c is limited by the erythrocyte's life span of approximately 100 to 120 days. As a result, HbA1c reflects the average blood glucose level during the preceding 2 to 3 months. HbA1c is thus suitable to monitor long-term blood glucose control in individuals with diabetes mellitus.[3,4] Glucose levels closer to the time of the assay have a greater influence on the HbA1c level, since the plasma glucose in the preceding month determines 50 % of the HbA1c concentration, whereas days 60 to 120 determines only 25 %. HbA1c is relatively unaffected by recent acute fluctuations in glucose levels.[2]

The approximate relationship between HbA1c and mean blood glucose values was analyzed in several studies.[5,6,7] The following correlations have been described:

*According to IFCC standardization*[8]

• Estimated average glucose [mmol/L] = 0.146 x HbA1c (mmol/mol) + 0.834 or

• Estimated average glucose [mg/dL] = 2.64 x HbA1c (mmol/mol) + 15.03

*According to DCCT/NGSP standardization*[9]

• Estimated average glucose [mmol/L] = 1.59 x HbA1c (%) - 2.59 or

• Estimated average glucose [mg/dL] = 28.7 x HbA1c (%) - 46.7

With these reference systems, HbA1c results are reported globally in IFCC units (mmol/mol) and derived NGSP units (percent of total hemoglobin).

Fasting plasma glucose, two-hour plasma glucose during a 75 g oral glucose tolerance test (OGTT), or HbA1c may be used for diagnostic testing of diabetes mellitus. HbA1c testing every 2 to 6 months is recommended for monitoring of long-term glycemic control. In certain clinical situations, such as gestational diabetes, or after a major change in therapy, it may be useful to measure HbA1c more frequently than usual (e.g., monthly). Presence of impaired fasting glucose and/or impaired glucose tolerance and/or HbA1c levels slightly above normal reference ranges, define an increased risk for diabetes and cardiovascular disease (CVD). The risk of diabetic complications, such as diabetic nephropathy and retinopathy, increases with poor metabolic control. In accordance with its function as an indicator for the mean blood glucose level, HbA1c predicts the development of diabetic complications in diabetes patients.[10,11,12,13,14,15,16,17,18,19]

## Test principle[20,21,22]

This method uses TTAB* as the detergent in the hemolyzing reagent to eliminate interference from leukocytes (TTAB does not lyse leukocytes). Sample pretreatment to remove labile HbA1c is not necessary.

All hemoglobin variants which are glycated at the β-chain N-terminus and which have antibody-recognizable regions identical to that of HbA1c are determined by this assay. Consequently, the metabolic state of patients having uremia or the most frequent hemoglobinopathies (HbAS, HbAC, HbAE, HbAD) can be determined using this assay.[23,24,25]

*Tetradecyltrimethylammonium bromide

### Hemoglobin A1c

The HbA1c determination is based on the turbidimetric inhibition immunoassay (TINIA) for hemolyzed whole blood.

▪ Sample and addition of R1 (buffer/antibody):

Glycohemoglobin (HbA1c) in the sample reacts with anti-HbA1c antibody to form soluble antigen-antibody complexes. Since the specific HbA1c antibody site is present only once on the HbA1c molecule, formation of insoluble complexes does not take place.

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- Addition of R3 (buffer/polyhapten) and start of reaction:

  The polyhaptens react with excess anti-HbA1c antibodies to form an insoluble antibody-polyhapten complex which can be determined turbidimetrically.

**Hemoglobin**

Liberated hemoglobin in the hemolyzed sample is converted to a derivative having a characteristic absorption spectrum which is measured bichromatically during the preincubation phase (sample + R1) of the above immunological reaction. A separate Hb reagent is consequently not necessary.

The final result is expressed as mmol/mol HbA1c or % HbA1c and is calculated from the HbA1c/Hb ratio as follows:

**Protocol 1 (mmol/mol HbA1c acc. to IFCC):**

HbA1c (mmol/mol) = (HbA1c/Hb) × 1000

**Protocol 2 (% HbA1c acc. to DCCT/NGSP):**

HbA1c (%) = (HbA1c/Hb) × 91.5 + 2.15

**Reagents - working solutions**

**R1**   Antibody Reagent

MES buffer: 0.025 mol/L; TRIS buffer: 0.015 mol/L, pH 6.2; HbA1c antibody (ovine serum): ≥ 0.5 mg/mL; detergents; stabilizers; preservative

**R3**   Polyhapten Reagent

MES buffer: 0.025 mol/L; TRIS buffer: 0.015 mol/L, pH 6.2; HbA1c polyhapten: ≥ 8 µg/mL; detergents; stabilizers; preservative

R1 is in position B and R3 is in position C.

**Precautions and warnings**

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:



Warning

| | |
|---|---|
| H317 | May cause an allergic skin reaction. |

**Prevention:**

| | |
|---|---|
| P261 | Avoid breathing mist or vapours. |
| P272 | Contaminated work clothing should not be allowed out of the workplace. |
| P280 | Wear protective gloves. |

**Response:**

| | |
|---|---|
| P333 + P313 | If skin irritation or rash occurs: Get medical advice/attention. |
| P362 + P364 | Take off contaminated clothing and wash it before reuse. |

**Disposal:**

| | |
|---|---|
| P501 | Dispose of contents/container to an approved waste disposal plant. |

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

**Reagent handling**

Ready for use

**Storage and stability**

| | |
|---|---|
| Shelf life at 2-8 °C: | See expiration date on **cobas c** pack label. |
| On-board in use and refrigerated on the analyzer: | 4 weeks |

The reagents cannot be frozen. If freezing of a cassette is suspected a control measurement with this cassette is recommended.

**Specimen collection and preparation**

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Anticoagulated venous or capillary blood or hemolysate.

The only acceptable anticoagulants are Li-heparin, $K_2$-EDTA, $K_3$-EDTA, Fluoride/$Na_2$-EDTA, Na-Heparin and Fluoride/potassium oxalate.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

See the limitations and interferences section for details about possible sample interferences.

| | |
|---|---|
| Stability: | 3 days at 15-25 °C |
| | 7 days at 2-8 °C |
| | 6 months at (−15)-(−25) °C |

Freeze only once. Mix specimen thoroughly after thawing.

**Hemolysate preparation for Hemolysate Application**

*Manual hemolysate preparation:*

1. Allow blood specimen and Hemolyzing Reagent for Tina-quant HbA1c (Cat. No. 11488457122) to equilibrate at room temperature before use.

2. Moderately mix the sample immediately prior to pipetting, to ensure homogeneous mixture of erythrocytes. Take care to avoid the formation of foam.

3. Dilute the sample with Hemolyzing Reagent for Tina-quant HbA1c in the ratio 1:101 (1+100) using one of the following pipetting schemes.

   Pipette into tubes:

   Hemolyzing Reagent for Tina-quant HbA1c: **500 µL**
   Specimen (patient or control): **5 µL**
   or
   Hemolyzing Reagent for Tina-quant HbA1c: **1000 µL**
   Specimen (patient or control): **10 µL**
   or
   Hemolyzing Reagent for Tina-quant HbA1c: **2000 µL**
   Specimen (patient or control): **20 µL**

4. Mix using a vibration mixer or by gentle swirling.

5. The hemolysate can be used after the solution has changed color from red to brownish-green (approximately 1-2 min).

| | |
|---|---|
| Stability of the hemolysate: | 4 hours at 15-25 °C |
| | 24 hours at 2-8 °C |
| | 6 months at (−15)-(−25) °C |

Freeze only once. Mix specimen thoroughly after thawing.

**Materials provided**

See "Reagents – working solutions" section for reagents.

**Materials required (but not provided)**

See "Order information" section

General laboratory equipment

# A1CX3

**Tina-quant Hemoglobin A1cDx Gen.3**

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## Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

### Whole Blood application for Hb (HBW3) and HbA1c (A1W3)

#### Test definition Hb (HBW3)

| Reporting time | 10 min |
|---|---|
| Wavelength (sub/main) | 660/376 nm |

| Reagent pipetting | | Diluent ($H_2O$) |
|---|---|---|
| R1 | 76 µL | - |
| R3 | 15 µL | |

| Sample volumes | Sample | Sample dilution | |
|---|---|---|---|
| | | Sample | Diluent (Hemo-lyzing reagent) |
| Normal | 3.2 µL | 1.3 µL | 130 µL |
| Decreased | 3.2 µL | 1.3 µL | 130 µL |
| Increased | 3.2 µL | 1.3 µL | 130 µL |

#### Test definition HbA1c (A1W3)

| Reporting time | 10 min |
|---|---|
| Wavelength (sub/main) | 660/340 nm |

| Reagent pipetting | | Diluent ($H_2O$) |
|---|---|---|
| R1 | 76 µL | - |
| R3 | 15 µL | |

| Sample volumes | Sample | Sample dilution | |
|---|---|---|---|
| | | Sample | Diluent (Hemo-lyzing reagent) |
| Normal | 3.2 µL | 1.3 µL | 130 µL |
| Decreased | 3.2 µL | 1.3 µL | 130 µL |
| Increased | 3.2 µL | 1.3 µL | 130 µL |

#### Ratio definition for mmol/mol HbA1c and % HbA1c calculation

#### Protocol 1 (mmol/mol HbA1c acc. to IFCC):

| **Abbreviated ratio name** | **RWI3 (20667)** |
|---|---|
| Equation | (A1W3/HBW3) × 1000 |
| Unit | mmol/mol |

#### Protocol 2 (% HbA1c acc. to DCCT/NGSP):

| **Abbreviated ratio name** | **RWD3 (20662)** |
|---|---|
| Equation | (A1W3/HBW3) × 91.5 + 2.15 |
| Unit | % |

The protocols are already implemented in the application (ACNs 20667 and 20662). It is recommended to report % HbA1c values (DCCT/NGSP) to one decimal place and mmol/mol HbA1c values (IFCC) without decimal places.

### Hemolysate application for Hb (HBH3) and HbA1c (A1H3)

#### Test definition Hb (HBH3)

| Reporting time | 10 min |
|---|---|
| Wavelength (sub/main) | 660/376 nm |

| Reagent pipetting | | Diluent ($H_2O$) |
|---|---|---|
| R1 | 76 µL | - |

| R3 | 15 µL |
|---|---|

| Sample volumes | Sample | Sample dilution | |
|---|---|---|---|
| | | Sample | Diluent (Hemo-lyzing reagent) |
| Normal | 3.2 µL | - | - |
| Decreased | 3.2 µL | - | - |
| Increased | 3.2 µL | - | - |

#### Test definition HbA1c (A1H3)

| Reporting time | 10 min |
|---|---|
| Wavelength (sub/main) | 660/340 nm |

| Reagent pipetting | | Diluent ($H_2O$) |
|---|---|---|
| R1 | 76 µL | - |
| R3 | 15 µL | |

| Sample volumes | Sample | Sample dilution | |
|---|---|---|---|
| | | Sample | Diluent (Hemo-lyzing reagent) |
| Normal | 3.2 µL | - | - |
| Decreased | 3.2 µL | - | - |
| Increased | 3.2 µL | - | - |

#### Ratio definition for HbA1c (mmol/mol (IFCC) or % (DCCT/NGSP)) calculation

#### Protocol 1 (mmol/mol HbA1c acc. to IFCC):

| **Abbreviated ratio name** | **RHI3 (20666)** |
|---|---|
| Equation | (A1H3/HBH3) × 1000 |
| Unit | mmol/mol |

#### Protocol 2 (% HbA1c acc. to DCCT/NGSP):

| **Abbreviated ratio name** | **RHD3 (20665)** |
|---|---|
| Equation | (A1H3/HBH3) × 91.5 + 2.15 |
| Unit | % |

The protocols are already implemented in the application (ACNs 20666 and 20665). It is recommended to report % HbA1c values (DCCT/NGSP) to one decimal place and mmol/mol HbA1c values (IFCC) without decimal places.

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

### Calibration for Whole Blood and Hemolysate Application

**Hb**

| Calibrators | S1-S2: C.f.a.s. HbA1c |
|---|---|
| Calibration mode | Linear |

**HbA1c**

| Calibrators | S1-S6: C.f.a.s. HbA1c |
|---|---|
| Calibration mode | Non-linear |
| Calibration frequency | Hb: 2-point calibration is recommended |
| | HbA1c: full calibration is recommended |

- every 29 days during shelf life
- after reagent lot change
- as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

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**Tina-quant Hemoglobin A1cDx Gen.3**

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Always calibrate both assays (Hb and HbA1c) in parallel.

Traceability: This method has been standardized against the approved IFCC reference method for the measurement of HbA1c in human blood[26,27] and can be transferred to results traceable to DCCT/NGSP by calculation.

## Note for Whole Blood and Hemolysate Application

For these applications C.f.a.s. HbA1c calibrator values are reagent lot matched. For each application and each combination of C.f.a.s. HbA1c calibrator lot and Tina-quant Hemoglobin A1cDx Gen.3 reagent lot the exact calibrator values are given in the respective electronically available value sheet. The lot-specific calibrator values are automatically linked to the correct reagent lot via the software of the analyzer.

The **cobas c** pack A1CD (Hemolyzing Reagent, 50 mL), Cat. No. 08463107190, needs to be available on the analyzer otherwise the calibration cannot be performed.

## Quality control for Whole Blood and Hemolysate Application

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. It is recommended to perform quality control always after lot calibration and subsequently at least every 4 weeks. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

## Calculation for Whole Blood and Hemolysate Application

*Hb, HbA1c*

**cobas c** systems automatically calculate the analyte concentration of each sample in the unit mmol/L (g/dL).

Conversion factor: mmol/L × 1.61 = g/dL

*HbA1c* ratio calculation:

For calculation of the mmol/mol HbA1c value (IFCC) and the percent HbA1c value (DCCT/NGSP), refer to the **Test principle** and **Ratio definition for mmol/mol HbA1c and % HbA1c calculation** sections in this method sheet.

## Limitations - interference[23,24,28,29,30,31,32,33,34,35]

1. For diagnostic purposes, mmol/mol HbA1c values (IFCC) and % HbA1c values (DCCT/NGSP) should be used in conjunction with information from other diagnostic procedures and clinical evaluations.

2. The test is designed only for accurate and precise measurement of mmol/mol HbA1c (IFCC) and % HbA1c (DCCT/NGSP). The individual results for total Hb and HbA1c concentration should not be reported.

3. As a matter of principle, care must be taken when interpreting any HbA1c result from patients with Hb variants. Abnormal hemoglobins might affect the half life of the red cells or the in vivo glycation rates. In these cases even analytically correct results do not reflect the same level of glycemic control that would be expected in patients with normal hemoglobin.[33] Whenever it is suspected that the presence of an Hb variant (e.g. HbSS, HbCC or HbSC) affects the correlation between the HbA1c value and glycemic control, HbA1c must not be used for the diagnosis of diabetes mellitus.

4. Any cause of shortened erythrocyte survival or decrease in mean erythrocyte age will reduce exposure of erythrocytes to glucose with a consequent decrease in mmol/mol HbA1c values (IFCC) and % HbA1c values (DCCT/NGSP), even though the time-averaged blood glucose level may be elevated. Causes of shortened erythrocyte lifetime might be hemolytic anemia or other hemolytic diseases, homozygous sickle cell trait, pregnancy, recent significant or chronic blood loss, etc. Similarly, recent blood transfusions can alter the mmol/mol HbA1c values (IFCC) and % HbA1c values (DCCT/NGSP). Caution should be used when interpreting the HbA1c results from patients with these conditions. HbA1c must not be used for the diagnosis of diabetes mellitus in the presence of such conditions.

5. Glycated HbF is not detected by the assay as it does not contain the glycated β-chain that characterizes HbA1c. However, HbF is measured in the total Hb assay and as a consequence, specimens containing high amounts of HbF (> 7 %) may result in lower than expected mmol/mol HbA1c values (IFCC) and % HbA1c values (DCCT/NGSP).[24,35]

6. mmol/mol HbA1c values (IFCC) and % HbA1c values (DCCT/NGSP) are not suitable for the diagnosis of gestational diabetes.[36]

7. In very rare cases of rapidly evolving type 1 diabetes the increase of the HbA1c values might be delayed compared to the acute increase in glucose concentrations. In these conditions diabetes mellitus must be diagnosed based on plasma glucose concentrations and/or the typical clinical symptoms.[36]

Criterion: Recovery within ± 7 % of initial value.

Icterus:[32] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 μmol/L or 60 mg/dL).

Lipemia (Intralipid):[32] No significant interference up to an Intralipid concentration of 600 mg/dL. There is poor correlation between triglycerides concentration and turbidity.

Glycemia: No significant interference from glucose up to a concentration of 55.5 mmol/L (1000 mg/dL). A fasting sample is not required.

Rheumatoid factors: No significant interference from rheumatoid factors up to a concentration of 750 IU/mL.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[37,38]

Other: No cross reactions with HbA0, HbA1a, HbA1b, acetylated hemoglobin, carbamylated hemoglobin, glycated albumin and labile HbA1c were found for the anti-HbA1c antibodies used in this kit.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

A special wash with the Special Cell Cleaning Solution is performed automatically after the fifth usage of each cuvette. For this purpose the **cobas c** pack SCCS (Special Cell Cleaning Solution, 50 mL), Cat. No. 08463093190 needs to be available on the analyzer otherwise the washing cannot be performed.

## ACTION REQUIRED

**Special Wash Programming:** The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

## Limits and ranges
## Measuring range

Hemoglobin: 2.48-24.8 mmol/L

HbA1c: 0.186-1.61 mmol/L

This corresponds to a measuring range of 23-196 mmol/mol HbA1c (IFCC) and 4.2-20.1 % HbA1c (DCCT/NGSP) at a typical hemoglobin concentration of 8.2 mmol/L.

In rare cases of ">Test" flags which might occur with the use of the whole blood application, remix the whole blood sample and repeat the analysis with the same settings.

It is recommended to switch the auto rerun function off.

## Lower limits of measurement

*Limit of Blank and Limit of Detection*

Hemoglobin:

| | |
|---|---|
| Limit of Blank | = 0.31 mmol/L |
| Limit of Detection | = 0.62 mmol/L |

HbA1c:

| | |
|---|---|
| Limit of Blank | = 0.12 mmol/L |
| Limit of Detection | = 0.18 mmol/L |

The Limit of Blank and Limit of Detection were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

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**Tina-quant Hemoglobin A1cDx Gen.3**

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The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the sample concentration which leads with a probability of 95 % to a measurement result above the Limit of Blank.

### Expected values

**Protocol 1 (mmol/mol HbA1c acc. to IFCC):** 29-42 mmol/mol HbA1c[39]

**Protocol 2 (% HbA1c acc. to DCCT/NGSP):** 4.8-5.9 % HbA1c[39]

This reference range was obtained by measuring 482 well-characterized healthy individuals without diabetes mellitus. HbA1c levels higher than the upper end of this reference range are an indication of hyperglycemia during the preceding 2 to 3 months or longer. According to the recommendations of the American Diabetes Association values above 48 mmol/mol HbA1c (IFCC) or 6.5 % HbA1c (DCCT/NGSP) are suitable for the diagnosis of diabetes mellitus.[36,40] Patients with HbA1c values in the range of 39-46 mmol/mol HbA1c (IFCC) or 5.7-6.4 % HbA1c (DCCT/NGSP) may be at risk of developing diabetes.[36,40]

HbA1c levels may reach 195 mmol/mol (IFCC) or 20 % (DCCT/NGSP) or higher in poorly controlled diabetes. Therapeutic action is suggested at levels above 64 mmol/mol HbA1c (IFCC) or 8 % HbA1c (DCCT/NGSP). Diabetes patients with HbA1c levels below 53 mmol/mol (IFCC) or 7 % (DCCT/NGSP) meet the goal of the American Diabetes Association.[31,30]

HbA1c levels below the established reference range may indicate recent episodes of hypoglycemia, the presence of Hb variants, or shortened lifetime of erythrocytes.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

### Specific performance data

Representative performance data on the analyzers are given below. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

### Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer (data based on DCCT/NGSP values):

### Whole Blood Application:

| Repeatability | Mean % HbA1c | SD % HbA1c | CV % |
|---|---|---|---|
| PreciControl HbA1c norm | 5.54 | 0.04 | 0.7 |
| PreciControl HbA1c path | 11.3 | 0.06 | 0.5 |
| Human sample 1 | 4.90 | 0.04 | 0.9 |
| Human sample 2 | 6.53 | 0.03 | 0.4 |
| Human sample 3 | 7.29 | 0.03 | 0.5 |
| Human sample 4 | 8.33 | 0.05 | 0.5 |
| Human sample 5 | 12.5 | 0.06 | 0.5 |

| Intermediate precision | Mean % HbA1c | SD % HbA1c | CV % |
|---|---|---|---|
| PreciControl HbA1c norm | 5.54 | 0.06 | 1.1 |
| PreciControl HbA1c path | 11.3 | 0.09 | 0.8 |
| Human sample 1 | 4.89 | 0.06 | 1.3 |
| Human sample 2 | 6.67 | 0.05 | 0.7 |
| Human sample 3 | 7.46 | 0.05 | 0.7 |
| Human sample 4 | 8.33 | 0.08 | 0.9 |
| Human sample 5 | 12.8 | 0.09 | 0.7 |

### Hemolysate Application:

| Repeatability | Mean % HbA1c | SD % HbA1c | CV % |
|---|---|---|---|
| PreciControl HbA1c norm | 5.57 | 0.03 | 0.5 |
| PreciControl HbA1c path | 11.1 | 0.07 | 0.6 |
| Human sample 1 | 4.97 | 0.03 | 0.5 |
| Human sample 2 | 6.57 | 0.03 | 0.5 |
| Human sample 3 | 7.26 | 0.04 | 0.5 |
| Human sample 4 | 8.24 | 0.04 | 0.5 |
| Human sample 5 | 12.4 | 0.06 | 0.5 |

| Intermediate precision | Mean % HbA1c | SD % HbA1c | CV % |
|---|---|---|---|
| PreciControl HbA1c norm | 5.57 | 0.10 | 1.8 |
| PreciControl HbA1c path | 11.1 | 0.14 | 1.3 |
| Human sample 1 | 4.98 | 0.11 | 2.2 |
| Human sample 2 | 6.68 | 0.09 | 1.3 |
| Human sample 3 | 7.39 | 0.09 | 1.2 |
| Human sample 4 | 8.41 | 0.09 | 1.1 |
| Human sample 5 | 12.8 | 0.17 | 1.3 |

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s).

### Method comparison

Evaluation of method comparison data is according to former NGSP certification criteria. The mean difference between the two methods and the 95 % confidence intervals of the differences in the range from 4-10 % (DCCT/NGSP) are given. 95 % of the differences between the values obtained for individual samples with both methods fall within the range defined by the lower and upper 95 % confidence intervals of the differences.

### Whole Blood Application:

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a **cobas c** 503 analyzer using the Tina-quant Hemoglobin A1cDx Gen.3 reagent with the whole blood application (y) were compared to those determined using the Tina-quant Hemoglobin A1c Gen.3 reagent with the whole blood application on a **cobas c** 501 analyzer (x).

Sample size (n) = 151

Mean difference: -0.050 % HbA1c

Lower 95 % confidence interval of differences: -0.274 % HbA1c

Upper 95 % confidence interval of differences: 0.174 % HbA1c

The sample concentrations were between 4.55 % and 9.97 % HbA1c (DCCT/NGSP values).

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a **cobas c** 503 analyzer using the Tina-quant Hemoglobin A1cDx Gen.3 reagent with the whole blood application (y) were compared to those determined using the Tina-quant Hemoglobin A1cDx Gen.3 reagent with the whole blood application on a **cobas c** 513 analyzer (x).

Sample size (n) = 159

Mean difference: 0.052 % HbA1c

Lower 95 % confidence interval of differences: -0.190 % HbA1c

Upper 95 % confidence interval of differences: 0.294 % HbA1c

The sample concentrations were between 4.77 % and 9.97 % HbA1c (DCCT/NGSP values).

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a **cobas c** 303 analyzer using the Tina-quant Hemoglobin A1cDx Gen.3 reagent with the whole blood application (y) were compared to those determined using the Tina-quant Hemoglobin A1c Gen.3 reagent with the whole blood application on a **cobas c** 501 analyzer (x).

# A1CX3

**Tina-quant Hemoglobin A1cDx Gen.3**

**cobas®**

Sample size (n) = 145

Mean difference: -0.023 % HbA1c

Lower 95 % confidence interval of differences: -0.371 % HbA1c

Upper 95 % confidence interval of differences: 0.324 % HbA1c

The sample concentrations were between 4.83 % and 9.93 % HbA1c (DCCT/NGSP values).

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a **cobas c** 303 analyzer using the Tina-quant Hemoglobin A1cDx Gen.3 reagent with the whole blood application (y) were compared to those determined using the Tina-quant Hemoglobin A1cDx Gen.3 reagent with the whole blood application on a **cobas c** 503 analyzer (x).

Sample size (n) = 147

Mean difference: 0.012 % HbA1c

Lower 95 % confidence interval of differences: -0.162 % HbA1c

Upper 95 % confidence interval of differences: 0.185 % HbA1c

The sample concentrations were between 4.67 % and 9.97 % HbA1c (DCCT/NGSP values).

**Hemolysate Application:**

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a **cobas c** 503 analyzer using the Tina-quant Hemoglobin A1cDx Gen.3 reagent with the hemolysate application (y) were compared to those determined using the Tina-quant Hemoglobin A1c Gen.3 reagent with the hemolysate application on a **cobas c** 501 analyzer (x).

Sample size (n) = 157

Mean difference: 0.037 % HbA1c

Lower 95 % confidence interval of differences: -0.311 % HbA1c

Upper 95 % confidence interval of differences: 0.385 % HbA1c

The sample concentrations were between 4.38 % and 9.94 % HbA1c (DCCT/NGSP values).

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a **cobas c** 503 analyzer using the Tina-quant Hemoglobin A1cDx Gen.3 reagent with the hemolysate application (y) were compared to those determined using the Tina-quant Hemoglobin A1cDx Gen.3 reagent with the hemolysate application on a **cobas c** 513 analyzer (x).

Sample size (n) = 160

Mean difference: 0.083 % HbA1c

Lower 95 % confidence interval of differences: -0.038 % HbA1c

Upper 95 % confidence interval of differences: 0.203 % HbA1c

The sample concentrations were between 4.72 % and 9.98 % HbA1c (DCCT/NGSP values).

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a **cobas c** 303 analyzer using the Tina-quant Hemoglobin A1cDx Gen.3 reagent with the hemolysate application (y) were compared to those determined using the Tina-quant Hemoglobin A1c Gen.3 reagent with the hemolysate application on a **cobas c** 501 analyzer (x).

Sample size (n) = 148

Mean difference: 0.161 % HbA1c

Lower 95 % confidence interval of differences: -0.116 % HbA1c

Upper 95 % confidence interval of differences: 0.438 % HbA1c

The sample concentrations were between 4.45 % and 9.87 % HbA1c (DCCT/NGSP values).

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a **cobas c** 303 analyzer using the Tina-quant Hemoglobin A1cDx Gen.3 reagent with the hemolysate application (y) were compared to those determined using the Tina-quant Hemoglobin A1cDx Gen.3 reagent with the hemolysate application on a **cobas c** 503 analyzer (x).

Sample size (n) = 148

Mean difference: 0.178 % HbA1c

Lower 95 % confidence interval of differences: -0.038 % HbA1c

Upper 95 % confidence interval of differences: 0.393 % HbA1c

The sample concentrations were between 4.77 % and 9.72 % HbA1c (DCCT/NGSP values).

**Analytical specificity**

| | |
|---|---|
| Hb derivatives | Labile HbA1c (pre-HbA1c), acetylated Hb, and carbamylated Hb do not affect the assay results. |
| Hb variants | Specimens containing high amounts of HbF (> 7 %) may yield lower than expected HbA1c results. |

**Please note**

According to the consensus statement of the American Diabetes Association (ADA), the European Association for the Study of Diabetes (EASD), the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and International Diabetes Federation (IDF) HbA1c results should be reported in parallel, both in mmol/mol (IFCC) and % (DCCT/NGSP) values.[41] In addition an HbA1c derived estimated average glucose concentration can be reported which can be calculated according to the equations given in the Summary section of this method sheet. Former % HbA1c (IFCC) values must not be used due to the risk of mix up / misinterpretation with the % HbA1c (DCCT/NGSP) values.

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

The Summary of Safety & Performance Report can be found here: https://ec.europa.eu/tools/eudamed

**Symbols**

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

| | |
|---|---|
| CONTENT | Contents of kit |
| → | Volume for reconstitution |
| GTIN | Global Trade Item Number |
| Rx only | For USA: Caution: Federal law restricts this device to sale by or on the order of a physician. |

CE 0123

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

Roche

# ACP2

**Acid Phosphatase Gen.2**

**cobas®**

## Order information

| REF | ℹ️ | CONTENT | | Analyzer(s) on which **cobas c** pack(s) can be used |
|---|---|---|---|---|
| 08101442190 | 08101442500 | Acid Phosphatase Gen.2<br>4 × 140 tests ACP2/140 tests NPP2 or<br>4 × 280 tests ACP2-T | System-ID 2005 001 | **cobas c** 303, **cobas c** 503,<br>**cobas c** 703 |

Materials required (but not provided):

| | | |
|---|---|---|
| 10759350190 | Calibrator f.a.s. (12 × 3 mL) | Code 20401 |
| 05117003190 | PreciControl ClinChem Multi 1 (20 × 5 mL) | Code 20391 |
| 05947626190 | PreciControl ClinChem Multi 1 (4 × 5 mL) | Code 20391 |
| 05117216190 | PreciControl ClinChem Multi 2 (20 × 5 mL) | Code 20392 |
| 05947774190 | PreciControl ClinChem Multi 2 (4 × 5 mL) | Code 20392 |

## English

## System information
**ACP2:** ACN 20050 (Total acid phosphatase)
**NPP2:** ACN 20051 (Non-prostatic acid phosphatase)
**ACP2-T:** ACN 20052 (Total acid phosphatase only)

## Intended use
In vitro test for the quantitative determination of acid phosphatase in human serum on **cobas c** systems.

## Summary
Measurement of the activity of acid phosphatase (ACP) in serum with this assay, is used to aid in the diagnosis and management of prostate cancer.

Acid phosphatases (ACPs) are a group of enzymes with optimal activity at a pH below 7.0 and can be differentiated according to their immunological properties, tissue distribution and subcellular localisation. To date, at least 5 different ACPs have been reported in human tissues. Lysosomal acid phosphatase is stored in the lysosomes of all body cells, while the highest concentrations of extralysosomal ACP activity occur in the prostate, bone (osteoclasts), spleen, platelets and erythrocytes. ACP activity in blood serum is usually distinguished into tartrate-resistant and tartrate-refractory.[1,2,3] A specific form of ACP sensitive to tartrate inhibition is the secretory prostatic acid phosphatase (PAP), which is normally secreted by prostate tissue. In prostate cancer, circulating levels of PAP are increased.[3,4] PAP has therefore extensively been used as a serum marker for prostate cancer until the introduction of the current gold standard prostate-specific antigen (PSA).[5] Serum PAP levels are particularly increased in individuals with metastatic prostate cancer and correlate with tumor stage. It has been suggested that PAP has clinical application in patient management, in predicting disease recurrence or monitoring the effects of treatment.[4,6] However, PSA is indicated as the preferred test for screening, monitoring and predicting prostate cancer outcomes. Presence or absence of malignant disease can only be confirmed with a prostate biopsy. A multi-parametric magnetic resonance imaging (mpMRI) is recommended before prostate biopsy to facilitate the targeting of suspected lesions.[7,8,9,10]

Activity of total acid phosphatase increases in pathologic conditions of increased osteolysis and bone remodeling, in case of bone metastasis and other types of malignancies, in Gaucher's and Niemann-Pick diseases. Prostatic and total acid phosphatase levels increase after prostate surgery, biopsy, manipulation or catheterization, in the presence of benign prostate hypertrophy, prostatitis and prostate infarction.[1,2,11,12,13] Increased PAP levels should not be considered an absolut test for malignancy and PAP results should always be interpreted in combination with the patient's medical history and further diagnostic evaluations.
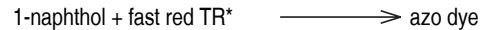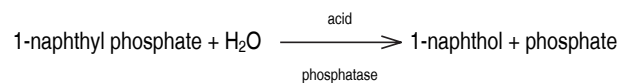
With this assay, PAP is detected with an indirect method by subtraction between ACP and non-prostatic acid phosphatase (NPP). The assay used here is a modification of the method described by Hillmann. Addition of 1,5-pentanediol increases the activity of prostatic acid phosphatase.[14]

## Test principle[14]
Colorimetric test
The 1-naphthol released during the enzymatic hydrolysis of 1-naphthyl phosphate is converted to an azo dye by coupling with diazotized fast red TR*. The tartrate is used as a specific inhibitor for prostatic acid phosphatase.

\* Fast red TR = 2-amino-5-chlorotoluene

$$1\text{-naphthyl phosphate} + H_2O \xrightarrow[\text{phosphatase}]{\text{acid}} 1\text{-naphthol} + \text{phosphate}$$

$$1\text{-naphthol} + \text{fast red TR*} \longrightarrow \text{azo dye}$$

## Reagents - working solutions

**R1**  Bottle R1:
Citrate buffer: 150 mmol/L, pH 4.8; 1,5-pentanediol: 220 mmol/L; detergent: 3.3 mL/L

Bottle R1a:
1-Naphthyl phosphate: 12.1 mmol/L; fast red TR salt: 1.2 mmol/L

Bottle R1b:
Sodium tartrate: 100 mmol/L (additionally for non-prostatic acid phosphatase determination)

**CH₃COOH**  Bottle 2:
Acetic acid: 0.8 mol/L (sample stabilizer)

## Precautions and warnings
For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:



Warning

| H373 | May cause damage to organs through prolonged or repeated exposure. |
|---|---|

**Prevention:**

| P260 | Do not breathe mist or vapours. |
|---|---|

**Response:**

| P314 | Get medical advice/attention if you feel unwell. |
|---|---|

# ACP2

**Acid Phosphatase Gen.2**

cobas®

**Disposal:**

P501      Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

## Reagent handling

*Total acid phosphatase*

Connect 1 bottle **R1** to 1 bottle **R1a** using the enclosed adapter and dissolve the substrate/chromogen mixture completely in the buffer. Fill the mixture into **cobas c** pack position B.

For ACP2-T prepare the total acid phosphatase reagent in duplicate as described above and fill 1 bottle of the mixture into **cobas c** pack position B and the other into **cobas c** pack position C so that both **cobas c** pack positions contain the same mixture.

*Non-prostatic acid phosphatase*

Connect 1 bottle **R1** to 1 bottle **R1a** using the enclosed adapter and dissolve the substrate/chromogen mixture completely in the buffer. Add a reagent tablet from bottle **R1b** and dissolve by gently swirling. Fill the mixture into **cobas c** pack position C.

## Storage and stability

| | |
|---|---|
| Shelf life at 2-8 °C: | See expiration date on **cobas c** pack label. |
| On-board in use and refrigerated on the analyzer: | 5 days |

## Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable. Serum

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Separate the serum from the clot or cells promptly.

Perform determinations on the samples immediately. Samples which cannot be examined immediately should be stabilized as follows: Add 1 drop (30 µL) of solution from bottle **2** to 1.0 mL of serum and mix.

Centrifuge samples containing precipitates before performing the assay.

See the limitations and interferences section for details about possible sample interferences.

| | |
|---|---|
| Stability:[15] | 8 days at 15-25 °C |
| | 8 days at 2-8 °C |
| | 4 months at -20 °C (±5 °C) |

Freeze only once.

## Materials provided

See "Reagents – working solutions" section for reagents.

## Materials required (but not provided)

See "Order information" section

General laboratory equipment

## Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

## Application for serum

### Test definition

*Total acid phosphatase and non-prostatic acid phosphatase*

| | | |
|---|---|---|
| Reporting time | 10 min | |
| Wavelength (sub/main) | 700/415 nm | |
| Reagent pipetting | | Diluent (H$_2$O) |
| R1 | 77 µL | – |

| *Sample volumes* | *Sample* | *Sample dilution* | |
|---|---|---|---|
| | | *Sample* | *Diluent (NaCl)* |
| Normal | 6.4 µL | – | – |
| Decreased | 2.1 µL | – | – |
| Increased | 6.4 µL | – | – |

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

### Calibration

*Total acid phosphatase:*

| | |
|---|---|
| Calibrators | S1: H$_2$O |
| | S2: C.f.a.s. |

*Non-prostatic acid phosphatase:*

| | |
|---|---|
| Calibrators | S1: H$_2$O |
| | S2: C.f.a.s. |
| Calibration mode | Linear |
| Calibration frequency | Full calibration<br>- after reagent lot change<br>- as required following quality control procedures |

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against the Roche system reagent using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ε.

### Quality control

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. It is recommended to perform quality control always after lot calibration and subsequently at least every 5 days. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

### Calculation

**cobas c** systems automatically calculate the analyte activity of each sample in the unit U/L (µkat/L).

Conversion factor: U/L × 0.0167 = µkat/L

A) Total acid phosphatase: See instrument printout.

B) Prostatic acid phosphatase:
The prostatic acid phosphatase can be determined manually by calculating the difference between the total acid phosphatase (ACP2) and the non-prostatic acid phosphatase (NPP2).

Activity $_{Prostatic\ acid\ phosphatase}$ =

     Activity $_{Total\ acid\ phosphatase}$ − Activity $_{Non-prostatic\ acid\ phosphatase}$

# ACP2

**Acid Phosphatase Gen.2**

<div align="right">

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</div>

## Limitations - interference

Criterion: Recovery within ±10 % of initial value at a total acid phosphatase activity of 7 U/L or at a non-prostatic acid phosphatase activity of 4 U/L.

Icterus:[16] No significant interference up to an I index of 1 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 17 µmol/L or 1 mg/dL).

Hemolysis:[16] No significant interference up to an H index of 200 (approximate hemoglobin concentration: 124 µmol/L or 200 mg/dL).

Lipemia (Intralipid):[16] No significant interference up to an L index of 200. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels. Exception: Methyldopa, cefoxitine and doxycycline cause artificially high non-prostatic acid phosphatase results.[17,18]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[19]

The addition of stabilizer to the sample interferes with the determination of other parameters.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

## ACTION REQUIRED

**Special Wash Programming:** The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

## Limits and ranges

### Measuring range

*Total acid phosphatase and non-prostatic acid phosphatase*

0.5-200 U/L (0.01-3.34 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:3 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 3.

### Lower limits of measurement

*Limit of Blank, Limit of Detection and Limit of Quantitation*

| | |
|---|---|
| Limit of Blank | = 0.5 U/L (0.01 µkat/L) |
| Limit of Detection | = 0.5 U/L (0.01 µkat/L) |
| Limit of Quantitation | = 0.5 U/L (0.01 µkat/L) |

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the activity below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low activity samples.

The Limit of Detection corresponds to the lowest analyte activity which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte activity that can be reproducibly measured with a total error of 20 %. It has been determined using low activity acid phosphatase samples.

## Expected values

### U/L

*Total acid phosphatase (37 °C)[20]*

| Men | < 6.6 U/L | Women | < 6.5 U/L |
|---|---|---|---|

*Prostatic acid phosphatase (37 °C)[20]*

| Men | < 3.5 U/L |
|---|---|

### µkat/L*

*Total acid phosphatase (37 °C)[20]*

| Men | < 0.110 µkat/L | Women | < 0.108 µkat/L |
|---|---|---|---|

*Prostatic acid phosphatase (37 °C)[20]*

| Men | < 0.058 µkat/L |
|---|---|

*calculated by unit conversion factor

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

## Specific performance data

Representative performance data on the analyzers are given below. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

## Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

*Total acid phosphatase*

| Repeatability | Mean U/L | SD U/L | CV % |
|---|---|---|---|
| PCCC1[a] | 26.2 | 0.0866 | 0.3 |
| PCCC2[b] | 48.4 | 0.182 | 0.4 |
| Human serum 1 | 1.39 | 0.0301 | 2.2 |
| Human serum 2 | 6.61 | 0.0495 | 0.7 |
| Human serum 3 | 38.4 | 0.135 | 0.4 |
| Human serum 4 | 101 | 0.477 | 0.5 |
| Human serum 5 | 181 | 0.748 | 0.4 |

| Intermediate precision | Mean U/L | SD U/L | CV % |
|---|---|---|---|
| PCCC1[a] | 26.3 | 0.287 | 1.1 |
| PCCC2[b] | 48.5 | 0.517 | 1.1 |
| Human serum 1 | 1.39 | 0.0477 | 3.4 |
| Human serum 2 | 6.73 | 0.0711 | 1.1 |
| Human serum 3 | 38.4 | 0.186 | 0.5 |
| Human serum 4 | 101 | 0.571 | 0.6 |
| Human serum 5 | 182 | 1.03 | 0.6 |

*Non-prostatic acid phosphatase*

| Repeatability | Mean U/L | SD U/L | CV % |
|---|---|---|---|
| PCCC1[a] | 13.8 | 0.0964 | 0.7 |
| PCCC2[b] | 29.0 | 0.174 | 0.6 |
| Human serum 1 | 1.30 | 0.0533 | 4.1 |
| Human serum 2 | 3.61 | 0.0455 | 1.3 |
| Human serum 3 | 51.2 | 0.186 | 0.4 |
| Human serum 4 | 99.4 | 0.393 | 0.4 |
| Human serum 5 | 172 | 0.787 | 0.5 |

| Intermediate precision | Mean U/L | SD U/L | CV % |
|---|---|---|---|

# ACP2

**Acid Phosphatase Gen.2**

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| | | | |
|---|---|---|---|
| PCCC1[a] | 14.0 | 0.190 | 1.4 |
| PCCC2[b] | 29.0 | 0.359 | 1.2 |
| Human serum 1 | 1.30 | 0.0672 | 5.2 |
| Human serum 2 | 3.61 | 0.0796 | 2.2 |
| Human serum 3 | 49.7 | 1.65 | 3.3 |
| Human serum 4 | 97.2 | 3.24 | 3.3 |
| Human serum 5 | 172 | 6.09 | 3.5 |

a) PreciControl ClinChem Multi 1
b) PreciControl ClinChem Multi 2

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s) and **cobas c** 703 analyzer(s).

**Method comparison**

Acid phosphatase values for human serum samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

*Total acid phosphatase:*
Sample size (n) = 62

| Passing/Bablok[21] | Linear regression |
|---|---|
| y = 1.010x + 0.169 U/L | y = 1.007x + 0.362 U/L |
| τ = 0.966 | r = 0.999 |

The sample activities were between 0.800 and 192 U/L.

*Non-prostatic acid phosphatase:*
Sample size (n) = 65

| Passing/Bablok[21] | Linear regression |
|---|---|
| y = 1.007x + 0.0189 U/L | y = 1.018x - 0.0518 U/L |
| τ = 0.961 | r = 1.000 |

The sample activities were between 0.700 and 196 U/L.

Acid phosphatase values for human serum samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

*Total acid phosphatase:*
Sample size (n) = 76

| Passing/Bablok[21] | Linear regression |
|---|---|
| y = 1.016x - 0.0375 U/L | y = 0.964x + 0.180 U/L |
| τ = 0.927 | r = 0.999 |

The sample activities were between 0.600 and 199 U/L.

*Non-prostatic acid phosphatase:*
Sample size (n) = 65

| Passing/Bablok[21] | Linear regression |
|---|---|
| y = 1.011x + 0.0644 U/L | y = 1.013x + 0.0408 U/L |
| τ = 0.948 | r = 1.000 |

The sample activities were between 0.800 and 192 U/L.

Acid phosphatase values for human serum samples obtained on a **cobas c** 703 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

*Total acid phosphatase:*
Sample size (n) = 81

| Passing/Bablok[21] | Linear regression |
|---|---|
| y = 0.995x + 0.120 U/L | y = 0.988x + 0.176 U/L |
| τ = 0.964 | r = 1.000 |

The sample activities were between 0.856 and 195 U/L.

*Non-prostatic acid phosphatase:*
Sample size (n) = 75

| Passing/Bablok[21] | Linear regression |
|---|---|
| y = 0.984x + 0.0846 U/L | y = 0.980x - 0.0572 U/L |
| τ = 0.790 | r = 0.999 |

The sample activities were between 0.588 and 195 U/L.

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

**Symbols**

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

| CONTENT | Contents of kit |
| → | Volume for reconstitution |
| GTIN | Global Trade Item Number |
| Rx only | For USA: Caution: Federal law restricts this device to sale by or on the order of a physician. |

CE 0123

# ALB2

**Albumin Gen.2**

## Order information

| REF | CONTENT | | Analyzer(s) on which **cobas c** pack(s) can be used |
|---|---|---|---|
| 08056692190 | Albumin Gen.2 (750 tests) | System-ID 2009 001 | **cobas c** 303, **cobas c** 503, **cobas c** 703 |

Materials required (but not provided):

| | | | |
|---|---|---|---|
| 10759350190 | Calibrator f.a.s. (12 x 3 mL) | Code 20401 | |
| 05117003190 | PreciControl ClinChem Multi 1 (20 x 5 mL) | Code 20391 | |
| 05947626190 | PreciControl ClinChem Multi 1 (4 x 5 mL) | Code 20391 | |
| 05117216190 | PreciControl ClinChem Multi 2 (20 x 5 mL) | Code 20392 | |
| 05947774190 | PreciControl ClinChem Multi 2 (4 x 5 mL) | Code 20392 | |
| 08063494190 | Diluent NaCl 9 % (123 mL) | System-ID 2906 001 | |

## English

### System information
**ALB2-G:** ACN 20090

### Intended use
In vitro test for the quantitative determination of albumin in human serum and plasma on **cobas c** systems.

### Summary[1,2]
Albumin is a carbohydrate-free protein, which constitutes 55-65 % of total plasma protein. It maintains plasma oncotic pressure, and is also involved in the transport and storage of a wide variety of ligands and is a source of endogenous amino acids. Albumin binds and solubilizes various compounds, e.g. bilirubin, calcium and long-chain fatty acids. Furthermore, albumin is capable of binding toxic heavy metal ions as well as numerous pharmaceuticals, which is the reason why lower albumin concentrations in blood have a significant effect on pharmacokinetics.

Hyperalbuminemia is of little diagnostic significance except in the case of dehydration. Hypoalbuminemia occurs during many illnesses and is caused by several factors: compromised synthesis due either to liver disease or as a consequence of reduced protein uptake; elevated catabolism due to tissue damage (severe burns) or inflammation; malabsorption of amino acids (Crohn's disease); proteinuria as a consequence of nephrotic syndrome; protein loss via the stool (neoplastic disease). In severe cases of hypoalbuminemia, the maximum albumin concentration of plasma is 2.5 g/dL (380 µmol/L). Due to the low osmotic pressure of the plasma, water permeates through blood capillaries into tissue (edema). The determination of albumin allows monitoring of a controlled patient dietary supplementation and serves also as an excellent test of liver function.

### Test principle[3]
Colorimetric assay

At a pH value of 4.1, albumin displays a sufficiently cationic character to be able to bind with bromcresol green (BCG), an anionic dye, to form a blue-green complex.

$$\text{Albumin} + \text{BCG} \xrightarrow{\text{pH 4.1}} \text{albumin-BCG complex}$$
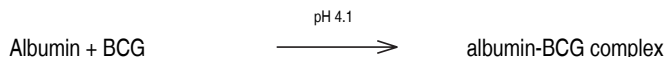
The color intensity of the blue-green color is directly proportional to the albumin concentration in the sample and is measured photometrically.

### Reagents - working solutions

**R1** Citrate buffer: 95 mmol/L, pH 4.1; preservatives, stabilizers

**R3** Citrate buffer: 95 mmol/L, pH 4.1; bromcresol green: 0.66 mmol/L; preservatives, stabilizers

R1 is in position B and R3 is in position C.

### Precautions and warnings
For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.
Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

### Reagent handling
Ready for use

### Storage and stability

| | |
|---|---|
| Shelf life at 15-25 °C: | See expiration date on **cobas c** pack label. |
| On-board in use and refrigerated on the analyzer: | 26 weeks |

### Specimen collection and preparation
For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Serum.
Plasma: Li-heparin and $K_2$-EDTA plasma
Do not use fluoride plasma.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

See the limitations and interferences section for details about possible sample interferences.

| Stability:[4] | 2.5 months at 20-25 °C |
|---|---|
| | 5 months at 4-8 °C |
| | 4 months at -20 °C |

### Materials provided
See "Reagents – working solutions" section for reagents.

### Materials required (but not provided)
See "Order information" section
General laboratory equipment

### Assay
For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

### Application for serum and plasma

### Test definition

| | |
|---|---|
| Reporting time | 10 min |
| Wavelength (sub/main) | 505/570 nm |
| Reagent pipetting | Diluent ($H_2O$) |

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**Albumin Gen.2**

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| R1 | 80 µL | – |
|---|---|---|
| R3 | 16 µL | 24 µL |

| *Sample volumes* | *Sample* | *Sample dilution* | |
|---|---|---|---|
| | | *Sample* | *Diluent (NaCl)* |
| Normal | 1.6 µL | – | – |
| Decreased | 1.6 µL | 25 µL | 50 µL |
| Increased | 1.6 µL | – | – |

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

## Calibration

| Calibrators | S1: $H_2O$ |
|---|---|
| | S2: C.f.a.s. |
| Calibration mode | Linear |
| Calibration frequency | Automatic full calibration |
| | - after reagent lot change |
| | Full calibration |
| | - after 4 weeks on-board |
| | - as required following quality control procedures |

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against the reference preparation of the IRMM (Institute for Reference Materials and Measurements) BCR470/CRM470 (RPPHS - Reference Preparation for Proteins in Human Serum).[5]

## Quality control

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. It is recommended to perform quality control always after lot calibration and subsequently at least every 26 weeks.

Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

## Calculation

**cobas c** systems automatically calculate the analyte concentration of each sample in the unit g/L (µmol/L, g/dL).

Conversion factors:

$$g/L \times 15.2 = µmol/L$$
$$g/L \times 0.1 = g/dL$$

## Limitations - interference

Criterion: Recovery within ± 10 % of initial values at an albumin concentration of 35 g/L (532 µmol/L).

Icterus:[6] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[6] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[6] No significant interference up to an L index of 550. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[7,8]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[9]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Colorimetric methods used for the determination of Albumin may lead to falsely elevated test results in patients suffering from renal failure or insufficiency due to interference with other proteins. Immunoturbidimetric methods are less affected.

**ACTION REQUIRED**
**Special Wash Programming:** The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

## Limits and ranges
### Measuring range

2-60 g/L (30.4-912 µmol/L)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:3 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 3.

### Lower limits of measurement

*Limit of Blank, Limit of Detection and Limit of Quantitation*

| Limit of Blank | = 2 g/L (30.4 µmol/L) |
|---|---|
| Limit of Detection | = 2 g/L (30.4 µmol/L) |
| Limit of Quantitation | = 3 g/L (45.6 µmol/L) |

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a total error of 20 %. It has been determined using low concentration albumin samples.

## Expected values
### g/L

| Reference range study[10] | |
|---|---|
| Adults | 39.7-49.4 g/L |
| Consensus values[11] | |
| Adults | 35-52 g/L |

Reference intervals according to Tietz[12]

| Newborn | |
|---|---|
| 0-4 days | 28-44 g/L |
| Children | |
| 4 days-14 years | 38-54 g/L |
| 14-18 years | 32-45 g/L |

### µmol/L*
* calculated by unit conversion factor

| Reference range study[10] | |
|---|---|
| Adults | 603-751 µmol/L |
| Consensus values[11] | |
| Adults | 532-790 µmol/L |

Reference intervals according to Tietz[12]

| Newborn | |
|---|---|
| 0-4 days | 426-669 µmol/L |

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Children
| | |
|---|---|
| 4 days-14 years | 578-821 µmol/L |
| 14-18 years | 486-684 µmol/L |

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

## Specific performance data
Representative performance data on the analyzers are given below. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

## Precision
Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

| Repeatability | Mean g/L | SD g/L | CV % |
|---|---|---|---|
| PCCC1[a] | 33.9 | 0.270 | 0.8 |
| PCCC2[b] | 47.2 | 0.223 | 0.5 |
| Human serum 1 | 52.3 | 0.252 | 0.5 |
| Human serum 2 | 16.0 | 0.245 | 1.5 |
| Human serum 3 | 32.7 | 0.280 | 0.9 |
| Human serum 4 | 45.6 | 0.253 | 0.6 |
| Human serum 5 | 49.5 | 0.258 | 0.5 |

| Intermediate precision | Mean g/L | SD g/L | CV % |
|---|---|---|---|
| PCCC1[a] | 33.9 | 0.865 | 2.6 |
| PCCC2[b] | 48.9 | 0.878 | 1.8 |
| Human serum 1 | 52.3 | 0.656 | 1.3 |
| Human serum 2 | 16.0 | 1.00 | 6.2 |
| Human serum 3 | 32.7 | 0.878 | 2.7 |
| Human serum 4 | 45.6 | 0.767 | 1.7 |
| Human serum 5 | 51.4 | 0.696 | 1.4 |

a) PreciControl ClinChem Multi 1
b) PreciControl ClinChem Multi 2

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s) and **cobas c** 703 analyzer(s).

## Method comparison
Albumin values for human serum and plasma samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).
Sample size (n) = 142

| Passing/Bablok[13] | Linear regression |
|---|---|
| y = 0.987x + 1.75 g/L | y = 0.999x + 1.26 g/L |
| τ = 0.851 | r = 0.992 |

The sample concentrations were between 2.60 and 57.7 g/L.

Albumin values for human serum and plasma samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).
Sample size (n) = 72

| Passing/Bablok[13] | Linear regression |
|---|---|
| y = 1.004x + 0.719 g/L | y = 1.001x + 0.852 g/L |
| τ = 0.922 | r = 0.998 |

The sample concentrations were between 2.84 and 57.2 g/L.

Albumin values for human serum and plasma samples obtained on a **cobas c** 703 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).
Sample size (n) = 75

| Passing/Bablok[13] | Linear regression |
|---|---|
| y = 1.005x - 0.450 g/L | y = 1.003x - 0.376 g/L |
| τ = 0.971 | r = 0.999 |

The sample concentrations were between 3.97 and 58.8 g/L.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

## References
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## Symbols
Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see navifyportal.roche.com for definition of symbols used):

| CONTENT | Contents of kit |
|---|---|
| →→→ | Volume for reconstitution |

# ALB2

**Albumin Gen.2**

| GTIN | Global Trade Item Number |
|------|--------------------------|

Rx only — For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

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All other product names and trademarks are the property of their respective owners.

Additions, deletions or changes are indicated by a change bar in the margin.

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CE 0123

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Roche

08056757500V9.0

# ALP2

**Alkaline phosphatase acc. to IFCC Gen.2**

cobas®

## Order information

| REF | 🔖 | CONTENT | | Analyzer(s) on which **cobas c** pack(s) can be used |
|-----|---|---------|--|------------------------------------------------------|
| 08056757190* | 08056757500 | Alkaline Phosphatase acc. to IFCC Gen.2 (1100 tests) | System-ID 2011 001 | **cobas c** 303, **cobas c** 503, **cobas c** 703 |
| 08056757214* | 08056757500 | Alkaline Phosphatase acc. to IFCC Gen.2 (1100 tests) | System-ID 2011 001 | **cobas c** 303, **cobas c** 503, **cobas c** 703 |

Materials required (but not provided):

| | | |
|--|--|--|
| 10759350190 | Calibrator f.a.s. (12 x 3 mL) | Code 20401 |
| 05117003190 | PreciControl ClinChem Multi 1 (20 x 5 mL) | Code 20391 |
| 05947626190 | PreciControl ClinChem Multi 1 (4 x 5 mL) | Code 20391 |
| 05117216190 | PreciControl ClinChem Multi 2 (20 x 5 mL) | Code 20392 |
| 05947774190 | PreciControl ClinChem Multi 2 (4 x 5 mL) | Code 20392 |
| 08063494190 | Diluent NaCl 9 % (123 mL) | System-ID 2906 001 |

* Some kits shown may not be available in all countries.

## English

### System information
**ALP2:** ACN 20110

### Intended use
In vitro test for the quantitative determination of alkaline phosphatase in human serum and plasma on **cobas c** systems.

### Summary
Measurement of alkaline phosphatase with this assay in human serum and plasma is used to aid in the diagnosis and monitoring of liver diseases and bone diseases.

Alkaline phosphatases (EC 3.1.3.1) are membrane-bound ectoenzymes that catalyze the hydrolysis of monophosphates from ester linkage under alkaline conditions (pH 8 to 10).[1] Alkaline phosphatase isoforms are encoded by 4 different genes: the liver-bone-kidney (tissue-nonspecific) variant, the intestinal variant, the placental variant and the variant from the germ cells (placental-like).[1,2] Alkaline phosphatase activity is present in various tissues, but its concentration varies, and the highest concentrations are typically found in the liver and bone. Although the exact metabolic function of the enzyme is not yet understood, it appears that it is associated with lipid transport in the intestine, with the calcification process in bone, and with host defense through endotoxin dephosphorylation. Minimal amounts of intestinal alkaline phosphatase may also be present and are subjected to increase after a meal.[2]

Total serum alkaline phosphatase measurement is used extensively as a clinical indicator of liver and bone health.[1,2,3,4,5,6,7,8,9] Any form of biliary tree obstruction induces the synthesis of alkaline phosphatase by hepatocytes, therefore a rise in the alkaline phosphatase activity in serum occurs with all forms of cholestasis and particularly with obstructive jaundice.[2,3,4,5] It is also elevated in diseases of the skeletal system associated with increased osteoblastic activity, such as Paget's disease, hyperparathyroidism, rickets and osteomalacia, as well as with fractures and malignant tumors.[1,6,7,8,9,10] A physiologic rise in the alkaline phosphatase activity is sometimes seen in children and juveniles. It is caused by increased osteoblast activity following accelerated bone growth.[1,2,10]

Decreased total alkaline phosphatase activity is rarely found in human serum but can occur in hypophosphatasia, in multiple myeloma with osteolytic lesions, secondary to growth hormone deficiency or in hypoparathyroidism.[1,10]

The assay method was first described by King and Armstrong, modified by Ohmori, Bessey, Lowry and Brock and later improved by Hausamen et al.[11,12,13,14] In 2011 the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Scientific Division, Committee on Reference Systems of Enzymes (C-RSE) recommended a reference procedure for the determination of alkaline phosphatase using an optimized substrate concentration and 2-amino-2-methyl-1-propanol as buffer plus the cations magnesium and zinc at 37 °C.[15] This assay follows the recommendations of the IFCC, but was optimized for performance and stability.

### Test principle[15]
Colorimetric assay in accordance with a standardized method.
In the presence of magnesium and zinc ions, p-nitrophenyl phosphate is cleaved by phosphatases into phosphate and p-nitrophenol.

$$p\text{-nitrophenyl phosphate} + H_2O \xrightarrow{ALP} phosphate + p\text{-nitrophenol}$$
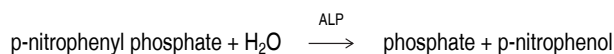
The p-nitrophenol released is directly proportional to the catalytic ALP activity. It is determined by measuring the increase in absorbance.

### Reagents - working solutions

**R1** 2-amino-2-methyl-1-propanol: 1.724 mol/L, pH 10.44 (30 °C); magnesium acetate: 3.83 mmol/L; zinc sulfate: 0.766 mmol/L; N-(2-hydroxyethyl)-ethylenediamine triacetic acid: 3.83 mmol/L

**R3** p-nitrophenyl phosphate: 132.8 mmol/L, pH 8.50 (25 °C); preservatives

R1 is in position B and R3 is in position C.

### Precautions and warnings
For in vitro diagnostic use for laboratory professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

| H315 | Causes skin irritation. |
|------|-------------------------|
| H319 | Causes serious eye irritation. |

**Prevention:**

| P264 | Wash skin thoroughly after handling. |
|------|--------------------------------------|
| P280 | Wear protective gloves/ eye protection/ face protection. |

# ALP2

**Alkaline phosphatase acc. to IFCC Gen.2**

**cobas®**

**Response:**

P302 + P352  IF ON SKIN: Wash with plenty of water.

P332 + P313  If skin irritation occurs: Get medical advice/attention.

P337 + P313  If eye irritation persists: Get medical advice/attention.

P362 + P364  Take off contaminated clothing and wash it before reuse.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

**Reagent handling**
Ready for use

**Storage and stability**

| | |
|---|---|
| Shelf life at 2-8 °C: | See expiration date on **cobas c** pack label. |
| On-board in use and refrigerated on the analyzer: | 8 weeks |

**Specimen collection and preparation**
For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Serum
Plasma: Li-heparin plasma

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

See the limitations and interferences section for details about possible sample interferences.

| Stability:[16] | 7 days at 20-25 °C |
|---|---|
| | 7 days at 4-8 °C |
| | 2 months at -20 °C (± 5 °C) |

Freeze only once.

**Materials provided**
See "Reagents – working solutions" section for reagents.

**Materials required (but not provided)**
See "Order information" section
General laboratory equipment

**Assay**
For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

**Application for serum and plasma**

**Test definition**

| Reporting time | 10 min | |
|---|---|---|
| Wavelength (sub/main) | 480/450 nm | |
| Reagent pipetting | | Diluent (H$_2$O) |
| R1 | 56 µL | 19 µL |
| R3 | 13 µL | 16 µL |
| *Sample volumes* | *Sample* | *Sample dilution* |
| | | *Sample*    *Diluent (NaCl)* |

| | | | |
|---|---|---|---|
| Normal | 2.1 µL | – | – |
| Decreased | 2.1 µL | 20 µL | 80 µL |
| Increased | 2.1 µL | – | – |

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

**Calibration**

| Calibrators | S1: H$_2$O |
|---|---|
| | S2: C.f.a.s. |
| Calibration mode | Linear |
| Calibration frequency | Full calibration |
| | - after reagent lot change |
| | - as required following quality control procedures |

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against the IFCC procedure (2011).[15]

**Quality control**
For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. It is recommended to perform quality control always after lot calibration and subsequently at least every 8 weeks. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

**Calculation**
**cobas c** systems automatically calculate the analyte activity of each sample in the unit U/L (µkat/L).

Conversion factor: U/L × 0.0167 = µkat/L

**Limitations - interference**
Criterion: Recovery within ± 10 U/L of initial values of samples ≤ 100 U/L and within ± 10 % for samples > 100 U/L.

Icterus:[17] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[17] No significant interference up to an H index of 200 (approximate hemoglobin concentration: 124 µmol/L or 200 mg/dL).

Lipemia (Intralipid):[17] No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[18,19]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[20]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

**ACTION REQUIRED**
**Special Wash Programming:** The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

# ALP2
**Alkaline phosphatase acc. to IFCC Gen.2**

**cobas®**

## Limits and ranges
**Measuring range**
5-1200 U/L (0.084-20.0 µkat/L)
Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 5.

**Lower limits of measurement**

*Limit of Blank, Limit of Detection and Limit of Quantitation*

| | |
|---|---|
| Limit of Blank | = 5 U/L (0.084 µkat/L) |
| Limit of Detection | = 5 U/L (0.084 µkat/L) |
| Limit of Quantitation | = 5 U/L (0.084 µkat/L) |

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the activity below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low activity samples.

The Limit of Detection corresponds to the lowest analyte activity which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte activity that can be reproducibly measured with a total error of 20 %. It has been determined using low activity alkaline phosphatase samples.

## Expected values

### U/L

Adults[21]

| | |
|---|---|
| Males (n = 221) | 40-129 U/L |
| Females (n = 229) | 35-104 U/L |

Children[22]

Males

| Age | | |
|---|---|---|
| | 0-14 days | 83-248 U/L |
| | 15 days -< 1 year | 122-469 U/L |
| | 1-< 10 years | 142-335 U/L |
| | 10-< 13 years | 129-417 U/L |
| | 13-< 15 years | 116-468 U/L |
| | 15-< 17 years | 82-331 U/L |
| | 17-< 19 years | 55-149 U/L |

Females

| Age | | |
|---|---|---|
| | 0-14 days | 83-248 U/L |
| | 15 days -< 1 year | 122-469 U/L |
| | 1-< 10 years | 142-335 U/L |
| | 10-< 13 years | 129-417 U/L |
| | 13-< 15 years | 57-254 U/L |
| | 15-< 17 years | 50-117 U/L |
| | 17-< 19 years | 45-87 U/L |

(measured at 37 °C)

### µkat/L*

Adults[21]

| | |
|---|---|
| Males (n = 221) | 0.67-2.15 µkat/L |
| Females (n = 229) | 0.58-1.74 µkat/L |

Children[22]

Males

| Age | | |
|---|---|---|
| | 0-14 days | 1.39-4.14 µkat/L |
| | 15 days -< 1 year | 2.04-7.83 µkat/L |
| | 1-< 10 years | 2.37-5.59 µkat/L |
| | 10-< 13 years | 2.15-6.96 µkat/L |
| | 13-< 15 years | 1.94-7.82 µkat/L |
| | 15-< 17 years | 1.37-5.53 µkat/L |
| | 17-< 19 years | 0.92-2.49 µkat/L |

Females

| Age | | |
|---|---|---|
| | 0-14 days | 1.39-4.14 µkat/L |
| | 15 days -< 1 year | 2.04-7.83 µkat/L |
| | 1-< 10 years | 2.37-5.59 µkat/L |
| | 10-< 13 years | 2.15-6.96 µkat/L |
| | 13-< 15 years | 0.95-4.24 µkat/L |
| | 15-< 17 years | 0.84-1.95 µkat/L |
| | 17-< 19 years | 0.75-1.45 µkat/L |

*calculated by unit conversion factor

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

## Specific performance data
Representative performance data on the analyzers are given below. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

**Precision**
Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

| *Repeatability* | *Mean* U/L | *SD* U/L | *CV* % |
|---|---|---|---|
| PCCC1[a] | 98.9 | 0.408 | 0.4 |
| PCCC2[b] | 223 | 0.673 | 0.3 |
| Human serum 1 | 10.2 | 0.319 | 3.1 |
| Human serum 2 | 36.2 | 0.293 | 0.8 |
| Human serum 3 | 144 | 0.645 | 0.4 |
| Human serum 4 | 606 | 1.27 | 0.2 |
| Human serum 5 | 1094 | 2.66 | 0.2 |

| *Intermediate precision* | *Mean* U/L | *SD* U/L | *CV* % |
|---|---|---|---|
| PCCC1[a] | 98.4 | 1.42 | 1.4 |
| PCCC2[b] | 223 | 2.83 | 1.3 |
| Human serum 1 | 9.27 | 1.08 | 11.6 |
| Human serum 2 | 35.3 | 1.21 | 3.4 |
| Human serum 3 | 144 | 1.63 | 1.1 |
| Human serum 4 | 607 | 3.30 | 0.5 |
| Human serum 5 | 1095 | 5.21 | 0.5 |

a) PreciControl ClinChem Multi 1
b) PreciControl ClinChem Multi 2

# ALP2

**Alkaline phosphatase acc. to IFCC Gen.2**

**cobas**®

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s) and **cobas c** 703 analyzer(s).

## Method comparison

Alkaline phosphatase values for human serum and plasma samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 88

| Passing/Bablok[23] | Linear regression |
|---|---|
| y = 0.987x - 1.24 U/L | y = 1.013x - 4.31 U/L |
| τ = 0.985 | r = 1.000 |

The sample activities were between 15.0 and 1171 U/L.

Alkaline phosphatase values for human serum and plasma samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 75

| Passing/Bablok[23] | Linear regression |
|---|---|
| y = 0.985x - 0.691 U/L | y = 0.996x - 3.04 U/L |
| τ = 0.994 | r = 1.000 |

The sample activities were between 15.8 and 1177 U/L.

Alkaline phosphatase values for human serum and plasma samples obtained on a **cobas c** 703 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

Sample size (n) = 75

| Passing/Bablok[23] | Linear regression |
|---|---|
| y = 1.010x + 0.171 U/L | y = 1.019x - 1.18 U/L |
| τ = 0.999 | r = 1.000 |

The sample concentrations were between 7.10 and 1129 U/L.

## References

1    Fraser WD, Alter DN. Bone and mineral metabolism. In: Rifai N, Chiu RWK, Young I, Burnham CAD, Wittwer CT, editors. Tietz Textbook of Laboratory Medicine, Saunders Elsevier, Philadelphia, 7th edition, 2023, chapter 54, p. 766-766.e85.

2    Panteghini M. Serum Enzymes. In: Rifai N, Chiu RWK, Young I, Burnham CAD, Wittwer CT, editors. Tietz Textbook of Laboratory Medicine, Saunders Elsevier, Philadelphia, 7th edition, 2023, chapter 32, p. 350-350.e36.

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6    Singer FR, Bone HG 3rd, Hosking DJ, et al. Endocrine Society. Paget's disease of bone: an endocrine society clinical practice guideline. J Clin Endocrinol Metab 2014 Dec;99(12):4408-4422.

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16   Guder WG, Narayanan S, Wisser H, et al. List of Analytes; Preanalytical Variables. Brochure in: Samples: From the Patient to the Laboratory. Darmstadt: GIT-Verlag 1996.

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18   Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.

19   Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.

20   Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

21   Abicht K, El-Samalouti V, Junge W, et al. Multicenter evaluation of new GGT and ALP reagents with new reference standardization and determination of 37 °C reference intervals. Clin Chem Lab Med 2001;39:Special Supplement pp S 346.

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23   Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

## Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

| | |
|---|---|
| CONTENT | Contents of kit |
| → | Volume for reconstitution |
| GTIN | Global Trade Item Number |
| Rx only | For USA: Caution: Federal law restricts this device to sale by or on the order of a physician. |

# ALP2

**Alkaline phosphatase acc. to IFCC Gen.2**

$\mathsf{C}\,\mathsf{E}$ 0123

Roche

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# ALTP2

**cobas®**

**Alanine Aminotransferase acc. to IFCC II**

## Order information

| REF | 📖ⓘ | CONTENT | | Analyzer(s) on which **cobas c** pack(s) can be used |
|---|---|---|---|---|
| 08104697190* | 08104697500 | Alanine Aminotransferase acc. to IFCC II (800 tests) | System-ID 2014 001 | **cobas c** 303, **cobas c** 503, **cobas c** 703 |
| 08104697214* | 08104697500 | Alanine Aminotransferase acc. to IFCC II (800 tests) | System-ID 2014 001 | **cobas c** 303, **cobas c** 503, **cobas c** 703 |

Materials required (but not provided):

| 10759350190 | Calibrator f.a.s. (12 x 3 mL) | Code 20401 | |
|---|---|---|---|
| 05117003190 | PreciControl ClinChem Multi 1 (20 x 5 mL) | Code 20391 | |
| 05947626190 | PreciControl ClinChem Multi 1 (4 x 5 mL) | Code 20391 | |
| 05117216190 | PreciControl ClinChem Multi 2 (20 x 5 mL) | Code 20392 | |
| 05947774190 | PreciControl ClinChem Multi 2 (4 x 5 mL) | Code 20392 | |
| 08063494190 | Diluent NaCl 9 % (123 mL) | System-ID 2906 001 | |

\* Some kits shown may not be available in all countries.

## English

### System information
**ALTP2:** ACN 20140

### Intended use
In vitro test for the quantitative determination of alanine aminotransferase (ALT) with pyridoxal phosphate activation in human serum and plasma on **cobas c** systems.

### Summary
Alanine aminotransferase (ALT) measurements, performed with this device, in human serum and plasma are used as an aid in diagnosis of hepatocellular injury and in monitoring chronic liver injury.

The enzyme alanine aminotransferase (ALT) is present in highest concentrations in the liver, in the cytosol of the hepatocytes, although it is also found in the kidney, and, in much smaller quantities, in heart and skeletal muscle cells.[1] ALT catalyzes the transfer of amino groups from L-alanine to α-ketoglutarate, resulting in L-glutamate and pyruvate. This is a critical process of the tricarboxylic acid cycle, in which the coenzyme pyridoxal phosphate (also known as pyridoxal-5-phosphate or active vitamin B6) is required. When liver injury occurs, ALT is released from injured liver cells and causes a significant serum elevation.[1]

Measurement of ALT activity is therefore used for the diagnosis of hepatic diseases such as acute and chronic viral hepatitis, nonalcoholic fatty liver disease (NAFLD), alcohol-related liver disease, ischemic hepatopathy, autoimmune hepatitis, biliary injury, suspected malignant infiltration, cholestasis.[1] Serum elevations of ALT activity are rarely observed in conditions other than parenchymal liver disease.[2] In addition, ALT testing is recommended for monitoring chronic hepatitis status and progression.[3]

Although both serum aspartate aminotransferase (AST) and ALT become elevated whenever disease processes affect liver cell integrity, evidence suggests that ALT is a more specific marker of hepatic injury than AST. Moreover, elevations of ALT activity persist longer than elevations of AST activity.[1,4]

In patients with vitamin B6 deficiency (insufficient endogenous pyridoxal phosphate), serum aminotransferase activity may be decreased. The addition of pyridoxal phosphate to this assay causes an increase in aminotransferase activity (activation higher for AST than for ALT) and prevents falsely low aminotransferase test results in these samples.[2]

### Test principle
This assay follows the recommendations of the IFCC, but was optimized for performance and stability.[5]

ALT catalyzes the transfer of an amino group between L-alanine and 2-oxoglutarate to form pyruvate and L-glutamate. The pyruvate then reacts with NADH in the presence of lactate dehydrogenase (LDH) to form L-lactate and NAD+. Pyridoxal phosphate serves as a coenzyme in the amino transfer reaction. It ensures full enzyme activation.

$$\text{L-Alanine + 2-oxoglutarate} \xrightarrow{\text{ALT}} \text{pyruvate + L-glutamate}$$

$$\text{Pyruvate + NADH + H}^+ \xrightarrow{\text{LDH}} \text{L-lactate + NAD}^+$$

The rate of the NADH oxidation is directly proportional to the catalytic ALT activity. It is determined by measuring the decrease in absorbance.

### Reagents - working solutions

**R1** TRIS buffer: 230 mmol/L, pH 7.15 (37 °C); L-alanine: 1140 mmol/L; LDH (microorganisms): ≥ 94 μkat/L; pyridoxamine phosphate: 0.23 mmol/L; albumin (bovine): 0.25 %; stabilizers; preservative

**R3** NADH: ≥ 0.71 mmol/L; 2-oxoglutarate: 96 mmol/L; preservative

R1 is in position B and R3 is in position C.

### Precautions and warnings
For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

### Reagent handling
Ready for use

### Storage and stability

| | |
|---|---|
| Shelf life at 2-8 °C: | See expiration date on **cobas c** pack label. |
| On-board in use and refrigerated on the analyzer: | 12 weeks |

### Specimen collection and preparation
For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Serum
Plasma: Li-heparin and K$_2$- and K$_3$-EDTA plasma

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

See the limitations and interferences section for details about possible sample interferences.

# ALTP2

**Alanine Aminotransferase acc. to IFCC II**

<div style="text-align: right;">

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</div>

Stability: 4 days at 15-25 °C

7 days at 2-8 °C

## Materials provided

See "Reagents – working solutions" section for reagents.

## Materials required (but not provided)

See "Order information" section

General laboratory equipment

## Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

## Application for serum and plasma

### Test definition

| Reporting time | 10 min | |
|---|---|---|
| Wavelength (sub/main) | 700/340 nm | |
| Reagent pipetting | | Diluent (H$_2$O) |
| R1 | 52 µL | 48 µL |
| R3 | 15 µL | – |

| Sample volumes | Sample | Sample dilution | |
|---|---|---|---|
| | | Sample | Diluent (NaCl) |
| Normal | 4.5 µL | – | – |
| Decreased | 4.5 µL | 10 µL | 90 µL |
| Increased | 4.5 µL | – | – |

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

### Calibration

| Calibrators | S1: H$_2$O |
|---|---|
| | S2: C.f.a.s. |
| Calibration mode | Linear |
| Calibration frequency | Automatic full calibration - after reagent lot change |
| | Full calibration - as required following quality control procedures |

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against the original IFCC formulation using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ε.[5]

### Quality control

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. It is recommended to perform quality control always after lot calibration and subsequently at least every 12 weeks. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

### Calculation

**cobas c** systems automatically calculate the analyte activity of each sample in the unit U/L (µkat/L).

Conversion factor: U/L x 0.0167 = µkat/L

## Limitations - interference

Criterion: Recovery within ± 4.0 U/L of initial values of samples ≤ 40 U/L and ± 10 % for samples > 40 U/L.

Icterus:[6] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[6] No significant interference up to an H index of 100 (approximate hemoglobin concentration: 62.2 µmol/L or 100 mg/dL). Contamination with erythrocytes will elevate results, because the analyte level in erythrocytes is higher than in normal sera. The level of interference may be variable depending on the content of analyte in the lysed erythrocytes.

Lipemia (Intralipid):[6] No significant interference up to an L index of 500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Lipemic samples may cause > Abs flagging.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[7,8]

Drug interferences are measured based on recommendations given in CLSI guidelines EP07 and EP37 and other published literature. Effects of concentrations exceeding these recommendations have not been characterized.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[9]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

## ACTION REQUIRED

**Special Wash Programming:** The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

## Limits and ranges

### Measuring range

5-700 U/L (0.08-11.7 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 10.

### Lower limits of measurement

*Limit of Blank, Limit of Detection and Limit of Quantitation*

| Limit of Blank | = 5 U/L (0.08 µkat/L) |
|---|---|
| Limit of Detection | = 5 U/L (0.08 µkat/L) |
| Limit of Quantitation | = 5 U/L (0.08 µkat/L) |

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the activity below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low activity samples.

The Limit of Detection corresponds to the lowest analyte activity which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte activity that can be reproducibly measured with a total error of 20 %. It has been determined using low activity alanine aminotransferase samples.

# ALTP2

**Alanine Aminotransferase acc. to IFCC II**

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## Expected values
### U/L

Acc. to IFCC/Standard Method 94 with pyridoxal phosphate activation measured at 37 °C:[10]

| | |
|---|---|
| Males: | 10-50 U/L |
| Females: | 10-35 U/L |

Consensus values with pyridoxal phosphate activation:[11]

| | |
|---|---|
| Males: | up to 50 U/L |
| Females: | up to 35 U/L |

### µkat/L*

Acc. to IFCC/Standard Method 94 with pyridoxal phosphate activation measured at 37 °C:[10]

| | |
|---|---|
| Males: | 0.17-0.84 µkat/L |
| Females: | 0.17-0.58 µkat/L |

Consensus values with pyridoxal phosphate activation:[11]

| | |
|---|---|
| Males: | up to 0.84 µkat/L |
| Females: | up to 0.58 µkat/L |

*calculated by unit conversion factor

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

## Specific performance data
Representative performance data on the analyzers are given below. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

## Precision
Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

| Repeatability | Mean U/L | SD U/L | CV % |
|---|---|---|---|
| PCCC1[a] | 49.4 | 0.534 | 1.1 |
| PCCC2[b] | 127 | 3.04 | 2.4 |
| Human serum 1 | 12.8 | 0.474 | 3.7 |
| Human serum 2 | 30.8 | 0.601 | 2.0 |
| Human serum 3 | 54.7 | 0.965 | 1.8 |
| Human serum 4 | 359 | 2.45 | 0.7 |
| Human serum 5 | 630 | 2.81 | 0.4 |

| Intermediate precision | Mean U/L | SD U/L | CV % |
|---|---|---|---|
| PCCC1[a] | 49.2 | 1.80 | 3.7 |
| PCCC2[b] | 127 | 4.96 | 3.9 |
| Human serum 1 | 12.8 | 0.611 | 4.8 |
| Human serum 2 | 30.8 | 0.818 | 2.7 |
| Human serum 3 | 54.7 | 1.58 | 2.9 |
| Human serum 4 | 359 | 3.28 | 0.9 |
| Human serum 5 | 638 | 5.07 | 0.8 |

a) PreciControl ClinChem Multi 1
b) PreciControl ClinChem Multi 2

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s) and **cobas c** 703 analyzer(s).

## Method comparison
ALT values for human serum and plasma samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using the test ALTLP on a **cobas c** 501 analyzer (x).

Sample size (n) = 100

| Passing/Bablok[12] | Linear regression |
|---|---|
| y = 0.993x + 1.52 U/L | y = 0.988x + 1.71 U/L |
| τ = 0.988 | r = 1.000 |

The sample activities were between 8.9 and 683 U/L.

ALT values for human serum and plasma samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

Sample size (n) = 50

| Passing/Bablok[12] | Linear regression |
|---|---|
| y = 1.036x - 0.787 U/L | y = 1.039x - 1.61 U/L |
| τ = 0.997 | r = 1.000 |

The sample activities were between 27.0 and 635 U/L.

ALT values for human serum and plasma samples obtained on a **cobas c** 703 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

Sample size (n) = 65

| Passing/Bablok[12] | Linear regression |
|---|---|
| y = 1.004x - 0.634 U/L | y = 1.003x - 0.635 U/L |
| τ = 0.974 | r = 1.000 |

The sample concentrations were between 6.07 and 647 U/L.

## References
1. Kim WR, Flamm SL, Di Bisceglie AM, et al. Public Policy Committee of the American Association for the Study of Liver Disease. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. Hepatology 2008 Apr;47(4):1363-1370. doi: 10.1002/hep.22109.

2. Panteghini M, Bais R. Amino Transferases. In: Burtis CA, Ashwood ER, Bruns DE. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 5th ed. 2012;573-576.

3. Guidelines for the Prevention, Care and Treatment of Persons with Chronic Hepatitis B Infection. Geneva: World Health Organization; 2015 Mar.

4. Kwo PY, Cohen SM, Lim JK. ACG Clinical Guideline: Evaluation of Abnormal Liver Chemistries. Am J Gastroenterol 2017 Jan;112(1):18-35. doi: 10.1038/ajg.2016.517.

5. Schumann G, Bonora R, Ceriotti F, et al. IFCC Primary Reference Procedures for the Measurement of Catalytic Activity Concentrations of Enzymes at 37 ° – Part 4. Reference Procedure for the Measurement of Catalytic Activity Concentration of Alanine Aminotransferase. Clin Chem Lab Med 2002;40(7):718-724. doi: 10.1515/CCLM.2002.124.

6. Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

7. Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.

8. Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.

9. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

# ALTP2

**Alanine Aminotransferase acc. to IFCC II**

10  Klauke R, Schmidt E, Lorentz K. Recommendations for carrying out standard ECCLS procedures (1988) for the catalytic concentrations of creatine kinase, aspartate aminotransferase, alanine aminotransferase and γ-glutamyltransferase at 37 °C. Eur J Clin Chem Clin Biochem 1993;31:901-909.

11  Thomas L, Müller M, Schumann G, et al. Consensus of DGKL and VDGH for interim reference intervals on enzymes in serum. J Lab Med 2005;29(5):301-308.

12  Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

**Symbols**

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see navifyportal.roche.com for definition of symbols used):

| CONTENT | Contents of kit |
| → | Volume for reconstitution |
| GTIN | Global Trade Item Number |
| Rx only | For USA: Caution: Federal law restricts this device to sale by or on the order of a physician. |

CE 0123

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

⟨Roche⟩

# AMYL2

α-Amylase EPS ver.2

**cobas**®

## Order information

| REF | 📖 i | CONTENT | | Analyzer(s) on which **cobas c** pack(s) can be used |
|---|---|---|---|---|
| 08056811190* | 08056811500 | α-Amylase EPS ver.2 (750 tests) | System-ID 2017 001 | **cobas c** 303, **cobas c** 503, **cobas c** 703 |
| 08056811214* | 08056811500 | α-Amylase EPS ver.2 (750 tests) | System-ID 2017 001 | **cobas c** 303, **cobas c** 503, **cobas c** 703 |

Materials required (but not provided):

| | | |
|---|---|---|
| 10759350190 | Calibrator f.a.s. (12 x 3 mL) | Code 20401 |
| 05117003190 | PreciControl ClinChem Multi 1 (20 x 5 mL) | Code 20391 |
| 05947626190 | PreciControl ClinChem Multi 1 (4 x 5 mL) | Code 20391 |
| 05117216190 | PreciControl ClinChem Multi 2 (20 x 5 mL) | Code 20392 |
| 05947774190 | PreciControl ClinChem Multi 2 (4 x 5 mL) | Code 20392 |
| 08063494190 | Diluent NaCl 9 % (123 mL) | System-ID 2906 001 |

\* Some kits shown may not be available in all countries.

## English

### System information

**AMYL2:** ACN 20170 (Serum/plasma)

**AMYL2U:** ACN 20171 (Urine)

### Intended use

In vitro test for the quantitative determination of α-amylase in human serum, plasma and urine on **cobas c** systems.

### Summary

Measurements of α-amylase in human serum, plasma and urine with this assay are used in conjunction with other parameters to aid in the diagnosis and management of pancreatic diseases, such as acute pancreatitis, in suspected patients.

The α-amylases (1,4-α-D-glucanohydrolases, EC 3.2.1.1) are digestive enzymes that catalyze the hydrolytic degradation of polymeric carbohydrates such as amylose, amylopectin and glycogen by cleaving 1,4-α-glucosidic bonds. Linear and branched polyglucans are hydrolyzed at different rates. End products for linear polyglucans (amylose) are maltose and some residual glucose; if branched-chain polyglucans are used as substrate, a residue of dextrins is formed in addition to maltose and glucose.[1]

Amylases are present in many organs and tissues. They are predominantly produced by salivary glands and pancreas and can be released into the digestive tract or transported to other organs via the bloodstream.[2] Due to its small size, amylase is able to pass through the glomeruli of the kidneys and is the only plasma enzyme normally found in the urine.[1] The two predominant types present in serum and urine are the pancreatic type (P-type) and the salivary type (S-type). The P-type is almost exclusively synthesized by the pancreas and the S-type is mainly secreted by the salivary glands.[1] Amylase activity is also found in tears, sweat, human milk, the lungs, thyroid, tonsils and the fallopian tube.[3]

Because of the sparsity of specific clinical symptoms of pancreatic diseases, α-amylase determinations are of considerable importance in pancreatic diagnostics. Elevated levels of amylase activities in serum or urine are characteristics of acute pancreatitis, and therefore they are mainly used in the diagnosis and monitoring of this disease.[4,5,6,7] Hyperamylasemia does not, however, only occur with acute pancreatitis, but also in renal failure (reduced glomerular filtration), tumors of the lungs or ovaries, pulmonary inflammation, diseases of the salivary gland, diabetic ketoacidosis, cerebral trauma, surgical interventions or in the case of macroamylasemia.[1,3,8,9,10,11,12]

Numerous methods have been described for the determination of α-amylase. These either determine the decrease in the amount of substrate viscometrically, turbidimetrically, nephelometrically and amyloclastically or measure the formation of degradation products saccharogenically or kinetically with the aid of enzyme-catalyzed subsequent reactions.[13,14] The kinetic method described here is based on the well-proven cleavage of 4,6-ethylidene-(G7)-1,4-nitrophenyl-(G1)-α,D-maltoheptaoside (Ethylidene Protected Substrate = EPS) by α-amylase and subsequent hydrolysis of all the degradation products to p-nitrophenol with the aid of α-glucosidase (100 % chromophore liberation).[15] The results of this method correlate with those obtained by HPLC. This assay follows the recommendation of the IFCC, but was optimized for performance and stability.[16]
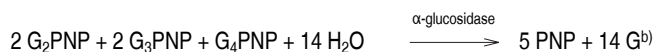
### Test principle[17,18]

Enzymatic colorimetric assay acc. to IFCC.

Defined oligosaccharides such as 4,6-ethylidene-$(G_7)$ p-nitrophenyl-$(G_1)$-α-D-maltoheptaoside (ethylidene-$G_7$PNP) are cleaved under the catalytic action of α-amylases. The $G_2$PNP, $G_3$PNP and $G_4$PNP fragments so formed are completely hydrolyzed to p-nitrophenol and glucose by α-glucosidase.

Simplified reaction scheme:

$$5 \text{ ethylidene-}G_7\text{PNP}^{a)} + 5 \text{ H}_2\text{O} \xrightarrow{\text{α-amylase}}$$
$$2 \text{ ethylidene-}G_5 + 2 \text{ } G_2\text{PNP} + 2 \text{ ethylidene-}G_4 + 2 \text{ } G_3\text{PNP} + \text{ ethylidene-}G_3 + G_4\text{PNP}$$

a) PNP ≙ p-nitrophenol

$$2 \text{ } G_2\text{PNP} + 2 \text{ } G_3\text{PNP} + G_4\text{PNP} + 14 \text{ H}_2\text{O} \xrightarrow{\text{α-glucosidase}} 5 \text{ PNP} + 14 \text{ } G^{b)}$$

b) G ≙ Glucose

The color intensity of the p-nitrophenol formed is directly proportional to the α-amylase activity. It is determined by measuring the increase in absorbance.

### Reagents - working solutions

**R1**  HEPES: 52.4 mmol/L; sodium chloride: 87 mmol/L; calcium chloride: 0.08 mmol/L; magnesium chloride: 12.6 mmol/L; α-glucosidase (microbial): ≥ 66.8 μkat/L; pH 7.0 (37 °C); preservatives; stabilizers

**R3**  HEPES: 52.4 mmol/L; ethylidene-$G_7$-PNP: 22 mmol/L; pH 7.0 (37 °C); preservatives; stabilizers

R1 is in position B and R3 is in position C.

### Precautions and warnings

For in vitro diagnostic use for laboratory professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

# AMYL2

**α-Amylase EPS ver.2**

**cobas®**



Warning

H317    May cause an allergic skin reaction.

**Prevention:**

P261    Avoid breathing mist or vapours.

P272    Contaminated work clothing should not be allowed out of the workplace.

P280    Wear protective gloves.

**Response:**

P333 + P313    If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364    Take off contaminated clothing and wash it before reuse.

**Disposal:**

P501    Dispose of contents/container to an approved waste disposal plant.

**Hazardous components:**

- 2-methyl-2H-isothiazol-3-one hydrochloride

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

**Reagent handling**

Ready for use

**Storage and stability**

| | |
|---|---|
| Shelf life at 2-8 °C: | See expiration date on **cobas c** pack label. |
| On-board in use and refrigerated on the analyzer: | 26 weeks |

**Specimen collection and preparation[16,19]**

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Serum
Plasma: Li-heparin plasma

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Urine: Collect urine without additives. α-Amylase is unstable in acid urine. Assay promptly or adjust pH to alkaline range (just above pH 7) before storage.[20]
If stabilizers are added to the sample, the sample index feature must not be used.

See the limitations and interferences section for details about possible sample interferences.

| | |
|---|---|
| Stability in *serum or plasma*:[20] | 7 days at 15-25 °C |
| | 1 month at 2-8 °C |
| Stability in *urine*:[21] | 2 days at 15-25 °C |
| | 10 days at 2-8 °C |

**Materials provided**

See "Reagents – working solutions" section for reagents.

**Materials required (but not provided)**

See "Order information" section
General laboratory equipment

**Assay**

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

**Application for serum, plasma and urine**

**Test definition**

| | |
|---|---|
| Reporting time | 10 min |
| Wavelength (sub/main) | 700/415 nm |

| Reagent pipetting | | Diluent (H$_2$O) |
|---|---|---|
| R1 | 78 µL | – |
| R3 | 16 µL | – |

| *Sample volumes* | *Sample* | *Sample dilution* | |
|---|---|---|---|
| | | *Sample* | *Diluent (NaCl)* |
| Normal | 3.1 µL | – | – |
| Decreased | 3.1 µL | 20 µL | 80 µL |
| Increased | 3.1 µL | – | – |

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

**Calibration**

*Application for serum/plasma (ACN 20170)*

| | |
|---|---|
| Calibrators | S1: H$_2$O |
| | S2: C.f.a.s. |
| Calibration mode | Linear |
| Calibration frequency | Automatic full calibration - after reagent lot change |
| | Full calibration - as required following quality control procedures |

*Application for urine (ACN 20171)*
Transfer of calibration from serum/plasma application (ACN 20170)

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against Roche system reagent using calibrated pipettes together with a manual photometer providing absolute values and substrate-specific absorptivity, ε.

**Quality control**

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

| | |
|---|---|
| Serum/plasma: | PreciControl ClinChem Multi 1 PreciControl ClinChem Multi 2 |
| Urine: | Quantitative urine controls are recommended for routine quality control. |

The control intervals and limits should be adapted to each laboratory's individual requirements. It is recommended to perform quality control always after lot calibration and subsequently at least every 26 weeks. Values obtained should fall within the defined limits. Each laboratory should

# AMYL2
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establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

## Calculation

**cobas c** systems automatically calculate the analyte activity of each sample in the unit U/L (µkat/L).

Conversion factor: $U/L \times 0.0167 = \mu kat/L$

## Limitations - interference

A slight change in the yellow coloration of solution 2 does not interfere with the performance of the test.

Do not pipette by mouth, and ensure that the reagent does not come into contact with the skin. **Saliva and sweat** contain α-amylase!

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[22]

### Serum/plasma

Criterion: Recovery within ± 10 U/L of initial values of samples ≤ 100 U/L and within ± 10 % for samples > 100 U/L.

Icterus:[23] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[23] No significant interference up to an H index of 500 (approximate hemoglobin concentration: 311 µmol/L or 500 mg/dL).

Lipemia (Intralipid):[23] No significant interference up to an L index of 1500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

In rare cases, samples with a combination of elevated turbidity (L-index) and high Amylase activity may cause a >React or >Abs flag.

Highly turbid and grossly lipemic samples may cause Abs. flags.

Anticoagulants: Interference was found with citrate, fluoride, and EDTA.[19]

Glucose: No significant interference from glucose up to a concentration of 111 mmol/L (2000 mg/dL). Approximately 10 % higher recovery was found at glucose concentrations of 250 mmol/L (4500 mg/dL).

Ascorbic acid: No significant interference from ascorbic acid up to a concentration of 5.68 mmol/L (100 mg/dL).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[24,25]
Exception: Icodextrin-based drugs may lead to decreased amylase results.[26]

### Urine

Criterion: Recovery within ± 46 U/L of initial values of samples ≤ 460 U/L and within ± 10 % for samples > 460 U/L.

Hemolysis: No significant interference up to an H index of 500 (approximate hemoglobin concentration: 311 µmol/L or 500 mg/dL).

Phosphate: No significant interference from phosphate up to a concentration of 70 mmol/L (217 mg/dL).

Urea: No significant interference from urea up to a concentration of 1500 mmol/L (9009 mg/dL).

Ascorbic acid: No significant interference from ascorbic acid up to a concentration of 2.27 mmol/L (40 mg/dL). Approximately 15 % lower recovery was found at ascorbic acid concentrations of 22.7 mmol/L (400 mg/dL).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[25]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

## ACTION REQUIRED
**Special Wash Programming:** The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

## Limits and ranges
### Measuring range
*Serum, plasma and urine*

3-1500 U/L (0.05-25.0 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 5.

### Lower limits of measurement
*Limit of Blank, Limit of Detection and Limit of Quantitation*

| | |
|---|---|
| Limit of Blank | = 3 U/L (0.05 µkat/L) |
| Limit of Detection | = 3 U/L (0.05 µkat/L) |
| Limit of Quantitation | = 3 U/L (0.05 µkat/L) |

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the activity below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low activity samples.

The Limit of Detection corresponds to the lowest analyte activity which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte activity that can be reproducibly measured with a total error of 20 %. It has been determined using low activity α-amylase samples.

### Expected values[16]

**U/L**

| | | |
|---|---|---|
| Serum/plasma | Men/Women | 28-100 U/L |
| Spontaneously voided urine | Men | 16-491 U/L |
| | Women | 21-447 U/L |
| α-amylase/ | Men | 58-283 U/g |
| creatinine quotient | Women | 75-390 U/g |

**µkat/L\***

| | | |
|---|---|---|
| Serum/plasma | Men/Women | 0.47-1.67 µkat/L |
| Spontaneously voided urine | Men | 0.27-8.20 µkat/L |
| | Women | 0.35-7.46 µkat/L |
| α-amylase/ | Men | 0.97-4.73 µkat/g |
| creatinine quotient | Women | 1.25-6.51 µkat/g |

*calculated by unit conversion factor

*α-Amylase/creatinine quotient*

To allow for fluctuations in the α-amylase activity in urine, it is advisable to determine the α-amylase/creatinine quotient. To do this, determine the α-amylase activity and creatinine concentration in spontaneously voided urine.

$$\text{Quotient [µkat/mmol or U/g]} = \frac{\alpha\text{-amylase [µkat/L or U/L]}}{\text{creatinine [mmol/L or g/L]}}$$

*Amylase/Creatinine Clearance Ratio (ACCR)[20]*

The ACCR is calculated from amylase activity and creatinine concentration. Both the serum and urine samples should be collected at the same time.

$$\text{ACCR [\%]} = \frac{\text{urine amylase [U/L]} \times \text{serum creatinine [mg/L]}}{\text{serum amylase [U/L]} \times \text{urine creatinine [mg/L]}} \times 100$$

The ACCR is approximately equal to 2-5 %.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

# AMYL2

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## Specific performance data

Representative performance data on the analyzers are given below. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

## Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

*Serum/plasma*

| Repeatability | Mean U/L | SD U/L | CV % |
|---|---|---|---|
| PCCC1[c] | 76.9 | 0.438 | 0.6 |
| PCCC2[d] | 193 | 0.831 | 0.4 |
| Human serum 1 | 7.38 | 0.231 | 3.1 |
| Human serum 2 | 63.9 | 0.345 | 0.5 |
| Human serum 3 | 509 | 1.63 | 0.3 |
| Human serum 4 | 771 | 2.67 | 0.3 |
| Human serum 5 | 1395 | 4.13 | 0.3 |

| Intermediate precision | Mean U/L | SD U/L | CV % |
|---|---|---|---|
| PCCC1[c] | 76.9 | 0.713 | 0.9 |
| PCCC2[d] | 194 | 1.51 | 0.8 |
| Human serum 1 | 7.38 | 0.263 | 3.6 |
| Human serum 2 | 63.6 | 0.409 | 0.6 |
| Human serum 3 | 509 | 2.51 | 0.5 |
| Human serum 4 | 771 | 4.13 | 0.5 |
| Human serum 5 | 1395 | 6.04 | 0.4 |

c) PreciControl ClinChem Multi 1
d) PreciControl ClinChem Multi 2

*Urine*

| Repeatability | Mean U/L | SD U/L | CV % |
|---|---|---|---|
| Control 1[e] | 56.3 | 0.327 | 0.6 |
| Control 2[e] | 180 | 0.707 | 0.4 |
| Human urine 1 | 7.78 | 0.257 | 3.3 |
| Human urine 2 | 263 | 0.913 | 0.3 |
| Human urine 3 | 408 | 1.13 | 0.3 |
| Human urine 4 | 766 | 1.96 | 0.3 |
| Human urine 5 | 1385 | 3.62 | 0.3 |

| Intermediate precision | Mean U/L | SD U/L | CV % |
|---|---|---|---|
| Control 1[e] | 56.3 | 0.370 | 0.7 |
| Control 2[e] | 180 | 0.801 | 0.4 |
| Human urine 1 | 7.74 | 0.403 | 5.2 |
| Human urine 2 | 263 | 2.09 | 0.8 |
| Human urine 3 | 409 | 10.6 | 2.6 |
| Human urine 4 | 767 | 4.41 | 0.6 |
| Human urine 5 | 1385 | 5.66 | 0.4 |

e) commercially available control material

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s) and **cobas c** 703 analyzer(s).

## Method comparison

Amylase values for human serum, plasma and urine samples obtained on a **cobas c** 503 analyzer (y) were compared to those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

*Serum/plasma*

Sample size (n) = 85

| Passing/Bablok[27] | Linear regression |
|---|---|
| y = 1.006x - 0.00259 U/L | y = 1.008x - 0.399 U/L |
| τ = 0.993 | r = 1.000 |

The sample activities were between 10.3 and 1439 U/L.

*Urine*

Sample size (n) = 67

| Passing/Bablok[27] | Linear regression |
|---|---|
| y = 0.997x + 0.221 U/L | y = 0.996x + 0.571 U/L |
| τ = 0.985 | r = 1.000 |

The sample activities were between 6.90 and 1467 U/L.

Amylase values for human serum, plasma and urine samples obtained on a **cobas c** 303 analyzer (y) were compared to those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

*Serum/plasma*

Sample size (n) = 73

| Passing/Bablok[27] | Linear regression |
|---|---|
| y = 1.013x - 0.271 U/L | y = 1.012x - 0.182 U/L |
| τ = 0.993 | r = 1.000 |

The sample activities were between 9.10 and 1460 U/L.

*Urine*

Sample size (n) = 71

| Passing/Bablok[27] | Linear regression |
|---|---|
| y = 1.014x - 0.186 U/L | y = 1.019x - 0.515 U/L |
| τ = 0.991 | r = 1.000 |

The sample activities were between 4.80 and 1444 U/L.

Amylase values for human serum, plasma and urine samples obtained on a **cobas c** 703 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

*Serum/plasma*

Sample size (n) = 73

| Passing/Bablok[27] | Linear regression |
|---|---|
| y = 1.009x + 0.257 U/L | y = 1.006x + 0.976 U/L |
| τ = 0.995 | r = 1.000 |

The sample concentrations were between 20.3 and 1412 U/L.

*Urine*

Sample size (n) = 75

| Passing/Bablok[27] | Linear regression |
|---|---|
| y = 0.993x - 0.0323 U/L | y = 0.992x + 0.111 U/L |
| τ = 0.994 | r = 1.000 |

The sample concentrations were between 5.20 and 1494 U/L.

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

## Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

| | |
|---|---|
| CONTENT | Contents of kit |
| → | Volume for reconstitution |
| GTIN | Global Trade Item Number |
| Rx only | For USA: Caution: Federal law restricts this device to sale by or on the order of a physician. |

CE 0123

Roche

# AMY-P

α-Amylase EPS pancreatic

## Order information

| REF | 📖ℹ | CONTENT | | Analyzer(s) on which **cobas c** pack(s) can be used |
|---|---|---|---|---|
| 08056820190 | 08056820500 | α-Amylase EPS Pancreatic (450 tests) | System-ID 2018 001 | **cobas c** 303, **cobas c** 503, **cobas c** 703 |

Materials required (but not provided):

| | | |
|---|---|---|
| 10759350190 | Calibrator f.a.s. (12 x 3 mL) | Code 20401 |
| 05117003190 | PreciControl ClinChem Multi 1 (20 x 5 mL) | Code 20391 |
| 05947626190 | PreciControl ClinChem Multi 1 (4 x 5 mL) | Code 20391 |
| 05117216190 | PreciControl ClinChem Multi 2 (20 x 5 mL) | Code 20392 |
| 05947774190 | PreciControl ClinChem Multi 2 (4 x 5 mL) | Code 20392 |
| 08063494190 | Diluent NaCl 9 % (123 mL) | System-ID 2906 001 |

## English

### System information

**AMYP2:** ACN 20180 (Serum/plasma)

**AMYP2U:** ACN 20181 (Urine)

### Intended use

In vitro test for the quantitative determination of pancreatic α-amylase in human serum, plasma and urine on **cobas c** systems.

### Summary

Measurements of pancreatic α-amylase in human serum, plasma and urine with this assay are used in conjunction with other parameters to aid in the diagnosis and management of pancreatic diseases, such as acute pancreatitis, in suspected patients.

The α-amylases (1,4-α-D-glucanohydrolases, EC 3.2.1.1) are digestive enzymes that catalyze the hydrolytic degradation of polymeric carbohydrates such as amylose, amylopectin and glycogen by cleaving 1,4-α-glucosidic bonds. Linear and branched polyglucans are hydrolyzed at different rates. End products for linear polyglucans (amylose) are maltose and some residual glucose; if branched-chain polyglucans are used as substrate, a residue of dextrins is formed in addition to maltose and glucose.[1]

Amylases are present in many organs and tissues. They are predominantly produced by salivary glands and pancreas and can be released into the digestive tract or transported to other organs via the bloodstream.[2] Due to its small size, amylase is able to pass through the glomeruli of the kidneys and is the only plasma enzyme normally found in the urine.[1] The two predominant types present in serum and urine are the pancreatic type (P-type) and the salivary type (S-type). The P-type is almost exclusively synthesized by the pancreas and the S-type is mainly secreted by the salivary glands.[1] Amylase activity is also found in tears, sweat, human milk, the lungs, thyroid, tonsils and the fallopian tube.[3]

Because of the sparsity of specific clinical symptoms of pancreatic diseases, enzymatic determinations are of considerable importance in pancreas diagnostics. The determination of pancreatic α-amylase is suitable for the diagnosis and monitoring of acute pancreatitis and may also have diagnostic value in chronic pancreatitis.[1,4,5,6,7,8,9,10,11] In studies of acute pancreatitis, the clinical sensitivity of measuring circulating pancreatic α-amylase level for diagnosing acute pancreatitis is consistently high, while the clinical specificity varies according to population and clinical case mix. However, when the pancreatic α-amylase measurement was evaluated in individuals with acute abdominal pain and suspected pancreatitis using a specific decision limit (three times the upper reference limit), it was found to be highly specific (above 90 %) in diagnosing acute pancreatitis. Therefore, pancreatic α-amylase has been described as more sensitive and specific for pancreatic tissue damage than total α-amylase.[12]

A variety of methods have been described for determining pancreatic α-amylase: radio- and enzyme-immunoassays as well as the partial inhibition of salivary α-amylase by an inhibitor derived from wheatgerm and calculation of the pancreatic α-amylase from the remaining and total amylase activities.[13,14,15,16,17]

The kinetic method described here is based on inhibition of the activity of human salivary α-amylase by two different monoclonal antibodies and the well-proven cleavage of 4,6-ethylidene-(G7)-1,4-nitrophenyl-(G1)-α-D-maltoheptaoside (Ethylidene Protected Substrate = EPS) by panc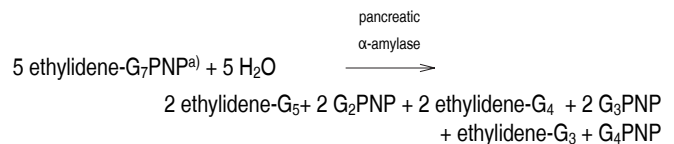reatic α-amylase followed by hydrolysis of all the degradation products to p-nitrophenol with the aid of α-glucosidase (100 % chromophore liberation). The results of this method correlate with those obtained by HPLC. This assay follows the recommendation of the IFCC, but was optimized for performance and stability.[18,19,20]

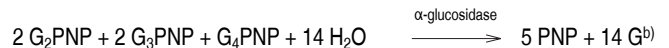### Test principle (simplified)[19,20]

Colorimetric assay

After immunoinhibition with antibodies against human salivary α-amylase the pancreatic α-amylase is selectively determined with an enzymatic colorimetric method using the substrate 4,6-ethylidene-p-nitrophenyl-α-D-maltoheptaoside (ethylidene-$G_7$PNP).[14]

Simplified reaction scheme:

$$5 \text{ ethylidene-}G_7PNP^{a)} + 5 H_2O \xrightarrow{\text{pancreatic } \alpha\text{-amylase}} 2 \text{ ethylidene-}G_5 + 2 G_2PNP + 2 \text{ ethylidene-}G_4 + 2 G_3PNP + \text{ethylidene-}G_3 + G_4PNP$$

a) PNP ≜ p-nitrophenol

$$2 G_2PNP + 2 G_3PNP + G_4PNP + 14 H_2O \xrightarrow{\alpha\text{-glucosidase}} 5 PNP + 14 G^{b)}$$

b) G ≜ Glucose

The rate of p-nitrophenol formation is directly proportional to the catalytic pancreatic α-amylase activity. It is determined by measuring the increase in absorbance photometrically.

### Reagents - working solutions

**R1** HEPES buffer: 52.4 mmol/L, pH 7.1 (37 °C); sodium chloride: 87 mmol/L; magnesium chloride: 12.6 mmol/L; calcium chloride: 0.075 mmol/L; α-glucosidase (microbial): ≥ 67 μkat/L; monoclonal antibodies (mouse): 97 mg/L; preservatives

**R3** HEPES buffer: 52.4 mmol/L, pH 7.1 (37 °C); 4,6-ethylidene-$G_7$ PNP: 22 mmol/L; preservatives; stabilizers

R1 is in position B and R3 is in position C.

### Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

# AMY-P

α-Amylase EPS pancreatic

**cobas®**



Warning

| | |
|---|---|
| H317 | May cause an allergic skin reaction. |

**Prevention:**

| | |
|---|---|
| P261 | Avoid breathing mist or vapours. |
| P272 | Contaminated work clothing should not be allowed out of the workplace. |
| P280 | Wear protective gloves. |

**Response:**

| | |
|---|---|
| P333 + P313 | If skin irritation or rash occurs: Get medical advice/attention. |
| P362 + P364 | Take off contaminated clothing and wash it before reuse. |

**Disposal:**

| | |
|---|---|
| P501 | Dispose of contents/container to an approved waste disposal plant. |

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

## Reagent handling
Ready for use

## Storage and stability

| | |
|---|---|
| Shelf life at 2-8 °C: | See expiration date on **cobas c** pack label. |
| On-board in use and refrigerated on the analyzer: | 26 weeks |

## Specimen collection and preparation[20,21]
For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Serum
Plasma: Li-heparin plasma

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Urine: Collect without additives. Pancreatic α-amylase is unstable in acid urine. Assay promptly or adjust pH to alkaline range (about pH 7) before storage.[22]
If stabilizers are added to the sample, the sample index feature must not be used.

See the limitations and interferences section for details about possible sample interferences.

| | |
|---|---|
| Stability in *serum or plasma*:[22] | 7 days at 15-25 °C |
| | 1 month at 2-8 °C |
| Stability in *urine*:[23] | 2 days at 15-25 °C |
| | 10 days at 2-8 °C |

## Materials provided
See "Reagents – working solutions" section for reagents.

## Materials required (but not provided)
See "Order information" section
General laboratory equipment

## Assay
For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

## Application for serum, plasma and urine

### Test definition

| | |
|---|---|
| Reporting time | 10 min |
| Wavelength (sub/main) | 700/415 nm |

| Reagent pipetting | | Diluent (H$_2$O) |
|---|---|---|
| R1 | 78 µL | – |
| R3 | 16 µL | – |

| *Sample volumes* | *Sample* | *Sample dilution* | |
|---|---|---|---|
| | | *Sample* | *Diluent (NaCl)* |
| Normal | 3.1 µL | – | – |
| Decreased | 3.1 µL | 20 µL | 80 µL |
| Increased | 3.1 µL | – | – |

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

### Calibration

*Application for serum/plasma (ACN 20180)*

| | |
|---|---|
| Calibrators | S1: H$_2$O |
| | S2: C.f.a.s. |
| Calibration mode | Linear |
| Calibration frequency | Automatic full calibration<br>- after reagent lot change |
| | Full calibration<br>- as required following quality control procedures |

*Application for urine (ACN 20181)*
Transfer of calibration from serum/plasma application (ACN 20180)

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against the Roche system reagent using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ε.

### Quality control
For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

| | |
|---|---|
| Serum/plasma: | PreciControl ClinChem Multi 1<br>PreciControl ClinChem Multi 2 |
| Urine: | Quantitative urine controls are recommended for routine quality control. |

The control intervals and limits should be adapted to each laboratory's individual requirements. It is recommended to perform quality control always after lot calibration and subsequently at least every 26 weeks. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

# AMY-P

### α-Amylase EPS pancreatic

**cobas**®

## Calculation

**cobas c** systems automatically calculate the analyte activity of each sample in the unit U/L (μkat/L).

Conversion factor: U/L × 0.0167 = μkat/L

### Limitations - interference[21,24]

The residual activity of salivary α-amylase is approx. 3 %. In rare cases, very high activities of salivary α-amylase can hence lead to elevated values being measured for pancreatic α-amylase.

A slight change in the yellow coloration of solution 2 does not interfere with the performance of the test.

Do not pipette by mouth, and ensure that the reagent does not come into contact with the skin. (**Saliva and sweat** contain α-amylase!)

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[25]

Patients with macroamylase may have elevated p-amylase results. The elevation is not due to an insufficient inhibition of salivary amylase in the serum immune complex. It is caused by a higher than normal level of p-amylase since the immune complex is not subject to glomerular filtration.

This elevated p-amylase is not diagnostic for pancreatitis. However, measurement of an elevated p-amylase in urine is confirmatory of pancreatitis, pancreatic trauma, or pancreatic carcinoma as the amylase released is not completely bound by the immune complex and thus subject to glomerular filtration.[26]

*Serum/plasma*

Criterion: Recovery within ± 5 U/L of initial values of samples ≤ 50 U/L and within ± 10 % for samples > 50 U/L.

Icterus:[27] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 μmol/L or 60 mg/dL).

Hemolysis:[27] No significant interference up to an H index of 200 (approximate hemoglobin concentration: 124 μmol/L or 200 mg/dL).

Lipemia (Intralipid):[27] No significant interference up to an L index of 1500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

In rare cases, samples with a combination of elevated turbidity (L-index) and high Amylase activity may cause a >React or >Abs flag.

Highly turbid and grossly lipemic samples may cause Abs. flags.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[28,29]

Exception: No interference from ascorbic acid up to 5.68 mmol/L (100 mg/dL). Icodextrin based drugs may lead to decreased amylase values.[26]

*Urine*

Criterion: Recovery within ± 35 U/L of initial values of samples ≤ 350 U/L and within ± 10 % for samples > 350 U/L.

Hemolysis: No significant interference up to an H index of 500 (approximate hemoglobin concentration: 311 μmol/L or 500 mg/dL).

Phosphate: No significant interference from phosphate up to a concentration of 60 mmol/L (186 mg/dL).

Urea: No significant interference from urea up to a concentration of 1500 mmol/L (9009 mg/dL).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[29]

Exception: Approximately 15 % lower recovery was found at ascorbic acid concentrations of 22.7 mmol/L (400 mg/dL).

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

## ACTION REQUIRED

**Special Wash Programming:** The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

## Limits and ranges

### Measuring range

*Serum, plasma and urine*

3-1500 U/L (0.05-25.0 μkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 5.

### Lower limits of measurement

*Limit of Blank, Limit of Detection and Limit of Quantitation*

| | |
|---|---|
| Limit of Blank | = 3 U/L (0.05 μkat/L) |
| Limit of Detection | = 3 U/L (0.05 μkat/L) |
| Limit of Quantitation | = 3 U/L (0.05 μkat/L) |

The Limit of Blank, the Limit of Detection and the Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the activity below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low activity samples.

The Limit of Detection corresponds to the lowest analyte activity which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte activity that can be reproducibly measured with a total error of 20 %. It has been determined using low activity α-amylase samples.

### Expected values[19]

**U/L**

| | | |
|---|---|---|
| Serum/plasma | Men/Women | 13-53 U/L |
| Spontaneously voided urine | Men | 7-356 U/L |
| | Women | 13-319 U/L |
| Pancreatic α-amylase/creatinine quotient | Men | 35-199 U/g |
| | Women | 52-259 U/g |

**μkat/L***

| | | |
|---|---|---|
| Serum/plasma | Men/Women | 0.22-0.88 μkat/L |
| Spontaneously voided urine | Men | 0.12-5.95 μkat/L |
| | Women | 0.22-5.33 μkat/L |
| Pancreatic α-amylase/creatinine quotient | Men | 0.58-3.33 μkat/g |
| | Women | 0.87-4.33 μkat/g |

*calculated by unit conversion factor

*Pancreatic α-amylase/creatinine quotient*

To allow for fluctuations in the pancreatic α-amylase activity in urine, it is advisable to determine the pancreatic α-amylase/creatinine quotient. To do this, determine the pancreatic α-amylase activity and creatinine concentration in spontaneously voided urine.

$$\text{Quotient [μkat/mmol or U/g]} = \frac{\text{pancreatic α-amylase [μkat/L or U/L]}}{\text{creatinine [mmol/L or g/L]}}$$

*Amylase/Creatinine Clearance Ratio (ACCR)[23]*

The ACCR is calculated from amylase activity and creatinine concentration. Both the serum and urine samples should be collected at the same time.

$$\text{ACCR [\%]} = \frac{\text{Urine amylase [U/L] × serum creatinine [mg/L]}}{\text{Serum amylase [U/L] × urine creatinine [mg/L]}} \times 100$$

ACCR approximately equal to 2-5 %.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

# AMY-P

α-Amylase EPS pancreatic

**cobas**®

## Specific performance data

Representative performance data on the analyzers are given below. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

### Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

*Serum/plasma*

| Repeatability | Mean U/L | SD U/L | CV % |
|---|---|---|---|
| PCCC1[c] | 38.3 | 0.314 | 0.8 |
| PCCC2[d] | 94.7 | 0.556 | 0.6 |
| Human serum 1 | 7.31 | 0.252 | 3.4 |
| Human serum 2 | 31.7 | 0.248 | 0.8 |
| Human serum 3 | 325 | 1.16 | 0.4 |
| Human serum 4 | 737 | 2.44 | 0.3 |
| Human serum 5 | 1254 | 3.80 | 0.3 |

| Intermediate precision | Mean U/L | SD U/L | CV % |
|---|---|---|---|
| PCCC1[c] | 38.3 | 0.358 | 0.9 |
| PCCC2[d] | 93.5 | 0.695 | 0.7 |
| Human serum 1 | 7.31 | 0.274 | 3.7 |
| Human serum 2 | 31.7 | 0.293 | 0.9 |
| Human serum 3 | 328 | 1.44 | 0.4 |
| Human serum 4 | 737 | 2.85 | 0.4 |
| Human serum 5 | 1254 | 5.05 | 0.4 |

c) PreciControl ClinChem Multi 1
d) PreciControl ClinChem Multi 2

*Urine*

| Repeatability | Mean U/L | SD U/L | CV % |
|---|---|---|---|
| Control 1 | 39.2 | 0.308 | 0.8 |
| Control 2 | 94.7 | 0.559 | 0.6 |
| Human urine 1 | 7.05 | 0.261 | 3.7 |
| Human urine 2 | 178 | 0.673 | 0.4 |
| Human urine 3 | 325 | 0.988 | 0.3 |
| Human urine 4 | 722 | 3.40 | 0.5 |
| Human urine 5 | 1311 | 6.66 | 0.5 |

| Intermediate precision | Mean U/L | SD U/L | CV % |
|---|---|---|---|
| Control 1 | 39.2 | 0.354 | 0.9 |
| Control 2 | 94.5 | 0.727 | 0.8 |
| Human urine 1 | 7.36 | 0.269 | 3.7 |
| Human urine 2 | 178 | 0.979 | 0.5 |
| Human urine 3 | 325 | 1.48 | 0.5 |
| Human urine 4 | 722 | 4.85 | 0.7 |
| Human urine 5 | 1311 | 7.41 | 0.6 |

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s) and **cobas c** 703 analyzer(s).

### Method comparison

Pancreatic amylase values for human serum, plasma and urine samples obtained on a **cobas c** 503 analyzer (y) were compared to those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

*Serum/plasma*

Sample size (n) = 88

| Passing/Bablok[30] | Linear regression |
|---|---|
| y = 1.005x + 0.0265 U/L | y = 1.002x + 0.381 U/L |
| τ = 0.983 | r = 1.000 |

The sample activities were between 3.80 and 1456 U/L.

*Urine*

Sample size (n) = 69

| Passing/Bablok[30] | Linear regression |
|---|---|
| y = 1.002x - 0.0394 U/L | y = 0.998x + 0.567 U/L |
| τ = 0.992 | r = 1.000 |

The sample activities were between 5.40 and 1440 U/L.

Pancreatic amylase values for human serum, plasma and urine samples obtained on a **cobas c** 303 analyzer (y) were compared to those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

*Serum/plasma*

Sample size (n) = 73

| Passing/Bablok[30] | Linear regression |
|---|---|
| y = 1.015x - 0.148 U/L | y = 1.014x - 0.174 U/L |
| τ = 0.988 | r = 1.000 |

The sample activities were between 7.30 and 1420 U/L.

*Urine*

Sample size (n) = 70

| Passing/Bablok[30] | Linear regression |
|---|---|
| y = 1.005x + 0.00463 U/L | y = 1.014x - 0.829 U/L |
| τ = 0.997 | r = 1.000 |

The sample activities were between 3.60 and 1441 U/L.

Pancreatic amylase values for human serum, plasma and urine samples obtained on a **cobas c** 703 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

*Serum/plasma*

Sample size (n) = 75

| Passing/Bablok[30] | Linear regression |
|---|---|
| y = 1.008x - 0.00402 U/L | y = 1.006x + 0.528 U/L |
| τ = 0.993 | r = 1.000 |

The sample concentrations were between 9.18 and 1467 U/L.

*Urine*

Sample size (n) = 73

| Passing/Bablok[30] | Linear regression |
|---|---|
| y = 0.997x - 0.312 U/L | y = 0.997x - 0.265 U/L |
| τ = 0.996 | r = 1.000 |

The sample concentrations were between 4.08 and 1395 U/L.

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

## Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

| CONTENT | Contents of kit |
| --- | --- |
| ⟶ | Volume for reconstitution |
| GTIN | Global Trade Item Number |
| Rx only | For USA: Caution: Federal law restricts this device to sale by or on the order of a physician. |

CE 0123

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⟨Roche⟩

# ASLOT

**cobas**®

**Tina-quant Antistreptolysin O**

## Order information

| REF | 📖i | CONTENT | | Analyzer(s) on which **cobas c** pack(s) can be used |
|---|---|---|---|---|
| 08105472190 | 08105472500 | Tina-quant Antistreptolysin O (200 tests) | System-ID 2021 001 | **cobas c** 303, **cobas c** 503, **cobas c** 703 |

Materials required (but not provided):

| | | |
|---|---|---|
| 03555941190 | C.f.a.s. PAC (3 × 1 mL) | Code 20589 |
| 10557897122 | Precinorm Protein (3 × 1 mL) | Code 20302 |
| 11333127122 | Precipath Protein (3 × 1 mL) | Code 20303 |
| 05117003190 | PreciControl ClinChem Multi 1 (20 × 5 mL) | Code 20391 |
| 05947626190 | PreciControl ClinChem Multi 1 (4 × 5 mL) | Code 20391 |
| 05117216190 | PreciControl ClinChem Multi 2 (20 × 5 mL) | Code 20392 |
| 05947774190 | PreciControl ClinChem Multi 2 (4 × 5 mL) | Code 20392 |
| 08063494190 | Diluent NaCl 9 % (123 mL) | System-ID 2906 001 |

## English

### System information
**ASLOT:** ACN 20210

### Intended use
In vitro test for the quantitative immunological determination of antistreptolysin O in human serum and plasma on **cobas c** systems.

### Summary
Antistreptolysin O (ASO) measurements, performed with this assay in human serum and plasma, are used as an aid in the diagnosis of antecedent group A streptococcus infection, which can be associated with post-infectious complications.

Group A streptococcus (GAS; Streptococcus pyogenes) is a Gram-positive, β-haemolytic bacterium which most commonly infects the throat or skin.[1,2] The ability of GAS to overcome innate and acquired immune mechanisms present in saliva allows the bacterium to remain viable for a long period.[1] Severe GAS infection results from the ability of the bacterium to migrate to normally sterile sites, such as the bloodstream and deep tissues.[1] Here, the interaction between host and pathogen factors leads to tissue destruction, bacterial dissemination and hyperinflammation.[1] Immunological defense reactions can be induced by several metabolites of the β-hemolyzing streptococci, which act as exogenous toxins for the human organism.[1] The most clinically important antibody reactions are found against streptolysin O, streptococcal deoxyribonuclease B, hyaluronidase and streptokinase. Determination of the antistreptolysin O antibody level is widely adopted to obtain useful information on preceding streptococcal infection.[2,3]

GAS is the cause of a wide range of acute, common pyogenic infections, including skin diseases or tonsillopharyngitis that may be followed by non-suppurative complications including acute rheumatic fever (ARF), rheumatic heart disease (RHD), poststreptococcal glomerulonephritis (PSGN), poststreptococcal reactive arthritis (PSRA) and pediatric autoimmune neuropsychiatric disorder associated with streptococcal infection (PANDAS).[2,3,4,5,6,7]

Early diagnosis, efficient treatment and monitoring of the patient can reduce risks and aid in management of post-infection complications.[8] Antistreptococcal antibody titers reflect past immunologic events. Antistreptolysin O titers begin to rise approximately 1 week and peak 3 to 6 weeks after the infection.[5] Ideally, to optimize diagnosis of preceding GAS infection, 2 sequential ASO measurements should be performed.[2,9] A fourfold or greater rise between 2 successive serological samples (10-14 days apart) in ASO titer is indicative of recent GAS infection.[9]

### Test principle[10,11,12,13]
Immunoturbidimetric assay

Human antistreptolysin O antibodies agglutinate with latex particles coated with streptolysin O antigens. The precipitate is determined turbidimetrically.

### Reagents - working solutions
**R1** TRIS buffer: 170 mmol/L, pH 8.2

**R3** Borate buffer: 10 mmol/L, pH 8.2; latex particles coated with streptolysin O: 2 mL/L

R1 is in position B and R3 is in position C.

### Precautions and warnings
For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Danger

| | |
|---|---|
| H317 | May cause an allergic skin reaction. |
| H360FD | May damage fertility. May damage the unborn child. |
| H412 | Harmful to aquatic life with long lasting effects. |

**Prevention:**

| | |
|---|---|
| P201 | Obtain special instructions before use. |
| P261 | Avoid breathing mist or vapours. |
| P273 | Avoid release to the environment. |
| P280 | Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection. |

**Response:**

| | |
|---|---|
| P308 + P313 | IF exposed or concerned: Get medical advice/attention. |
| P333 + P313 | If skin irritation or rash occurs: Get medical advice/attention. |

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

### Reagent handling
Ready for use

# ASLOT

**Tina-quant Antistreptolysin O**

cobas®

Carefully invert reagent container several times prior to use to ensure that the reagent components are mixed.

## Storage and stability

| | |
|---|---|
| Shelf life at 2-8 °C: | See expiration date on **cobas c** pack label. |
| On-board in use and refrigerated on the analyzer: | 26 weeks |

## Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Serum
Plasma: Li-heparin and $K_2$-EDTA plasma

The use of plasma can lead to a decrease in antistreptolysin O activity of approximately 7 %. For samples with an activity below 100 IU/mL the recovery in plasma can be either decreased or increased in comparison to serum.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

See the limitations and interferences section for details about possible sample interferences.

| | |
|---|---|
| Stability:[14] | 2 days at 20-25 °C |
| | 8 days at 4-8 °C |
| | 6 months at -20 °C (±5 °C) |

Freeze only once.

## Materials provided

See "Reagents – working solutions" section for reagents.

## Materials required (but not provided)

See "Order information" section
General laboratory equipment

## Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

## Application for serum

### Test definition

| | |
|---|---|
| Reporting time | 10 min |
| Wavelength (sub/main) | –/700 nm |
| Reagent pipetting | Diluent ($H_2O$) |
| R1 | 63 µL – |
| R3 | 63 µL – |

| Sample volumes | Sample | Sample dilution | |
|---|---|---|---|
| | | Sample | Diluent (NaCl) |
| Normal | 1.0 µL | – | – |
| Decreased | 1.0 µL | 20 µL | 102 µL |
| Increased | 1.0 µL | – | – |

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

## Calibration

| | |
|---|---|
| Calibrators | S1: $H_2O$ |
| | S2: C.f.a.s. PAC |
| Calibration mode | Linear |
| Calibration frequency | Automatic full calibration - after reagent lot change |
| | Full calibration - as required following quality control procedures |

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against an internal standard material.

## Quality control

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. It is recommended to perform quality control always after lot calibration and subsequently at least every 26 weeks. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

## Calculation

**cobas c** systems automatically calculate the analyte concentration of each sample in the unit IU/mL.

## Limitations - interference

Criterion: Recovery within ±10 % of initial value at an antistreptolysin O activity of 200 IU/mL.

Icterus:[15] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[15] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[15] No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors: No significant interference from rheumatoid factors up to a concentration of 180 IU/mL.

High-dose hook effect: No false result occurs up to an antistreptolysin O activity of 4000 IU/mL.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[16,17]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[18]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

## ACTION REQUIRED

**Special Wash Programming:** The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

## Limits and ranges
### Measuring range
20-600 IU/mL

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:6.1 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 6.1.

# ASLOT

**Tina-quant Antistreptolysin O**

**cobas®**

## Lower limits of measurement

*Limit of Blank, Limit of Detection and Limit of Quantitation*

| | |
|---|---|
| Limit of Blank | = 20 IU/mL |
| Limit of Detection | = 20 IU/mL |
| Limit of Quantitation | = 20 IU/mL |

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low activity samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a total error of 20 %. It has been determined using low activity antisptreptolysin O samples.

## Expected values[19]

| | |
|---|---|
| Adults | up to 200 IU/mL |
| Children | up to 150 IU/mL |

In some cases of streptococcal infections, particularly skin infections, there may be no observable increase in the ASO titer. As antistreptolysin O is only detectable in 85 % of all patients with rheumatic fever, the determination of anti-streptococcal deoxyribonuclease antibodies and anti-streptococcal hyaluronidase antibodies may also be necessary.[19]

An appropriate evaluation of streptococcal infection is possible only if the test is repeated after 1 or 2 weeks.[20] Both clinical and laboratory findings should be correlated in reaching a diagnosis.

ASO levels are age dependent and change with geographic location and with the local frequency of streptococcal infections.[21,22]

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

## Specific performance data

Representative performance data on the analyzers are given below. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

## Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

| Repeatability | Mean IU/mL | SD IU/mL | CV % |
|---|---|---|---|
| PCCC1[a] | 117 | 2.41 | 2.1 |
| PCCC2[b] | 251 | 2.44 | 1.0 |
| Human serum 1 | 47.0 | 1.56 | 3.3 |
| Human serum 2 | 86.7 | 3.49 | 4.0 |
| Human serum 3 | 190 | 2.50 | 1.3 |
| Human serum 4 | 307 | 3.37 | 1.1 |
| Human serum 5 | 527 | 4.91 | 0.9 |

| Intermediate precision | Mean IU/mL | SD IU/mL | CV % |
|---|---|---|---|
| PCCC1[a] | 121 | 2.87 | 2.4 |
| PCCC2[b] | 248 | 3.59 | 1.5 |
| Human serum 1 | 47.0 | 1.84 | 3.9 |
| Human serum 2 | 86.7 | 3.75 | 4.3 |
| Human serum 3 | 190 | 3.21 | 1.7 |
| Human serum 4 | 307 | 4.29 | 1.4 |
| Human serum 5 | 527 | 6.96 | 1.3 |

a) PreciControl ClinChem Multi 1
b) PreciControl ClinChem Multi 2

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s) and **cobas c** 703 analyzer(s).

## Method comparison

Antistreptolysin O values for human serum samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 68

| Passing/Bablok[23] | Linear regression |
|---|---|
| y = 1.047x - 9.04 IU/mL | y = 1.055x - 11.8 IU/mL |
| τ = 0.981 | r = 0.999 |

The sample concentrations were between 21 and 545 IU/mL.

Antistreptolysin O values for human serum samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 65

| Passing/Bablok[23] | Linear regression |
|---|---|
| y = 1.021x + 0.0368 IU/mL | y = 1.018x - 1.08 IU/mL |
| τ = 0.980 | r = 0.999 |

The sample concentrations were between 21.9 and 586 IU/mL.

Antistreptolysin O values for human serum samples obtained on a **cobas c** 703 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

Sample size (n) = 75

| Passing/Bablok[23] | Linear regression |
|---|---|
| y = 0.976x - 1.50 IU/mL | y = 0.979x - 1.66 IU/mL |
| τ = 0.987 | r = 0.999 |

The sample concentrations were between 36.4 and 593 IU/mL.

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

The Summary of Safety & Performance Report can be found here: https://ec.europa.eu/tools/eudamed

**Symbols**

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see navifyportal.roche.com for definition of symbols used):

| CONTENT | Contents of kit |
|---|---|

| → | Volume for reconstitution |
|---|---|
| GTIN | Global Trade Item Number |

| Rx only | For USA: Caution: Federal law restricts this device to sale by or on the order of a physician. |
|---|---|

CE 0123

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⬡ Roche

08104719500V9.0

# ASTP2

**Aspartate Aminotransferase acc. to IFCC II**

**cobas®**

## Order information

| REF | [i] | CONTENT | | Analyzer(s) on which **cobas c** pack(s) can be used |
|---|---|---|---|---|
| 08104719190* | 08104719500 | Aspartate Aminotransferase acc. to IFCC II (800 tests) | System-ID 2023 001 | **cobas c** 303, **cobas c** 503, **cobas c** 703 |
| 08104719214* | 08104719500 | Aspartate Aminotransferase acc. to IFCC II (800 tests) | System-ID 2023 001 | **cobas c** 303, **cobas c** 503, **cobas c** 703 |

Materials required (but not provided):

| | | | |
|---|---|---|---|
| 10759350190 | Calibrator f.a.s. (12 x 3 mL) | Code 20401 | |
| 05117003190 | PreciControl ClinChem Multi 1 (20 x 5 mL) | Code 20391 | |
| 05947626190 | PreciControl ClinChem Multi 1 (4 x 5 mL) | Code 20391 | |
| 05117216190 | PreciControl ClinChem Multi 2 (20 x 5 mL) | Code 20392 | |
| 05947774190 | PreciControl ClinChem Multi 2 (4 x 5 mL) | Code 20392 | |
| 08063494190 | Diluent NaCl 9 % (123 mL) | System-ID 2906 001 | |

* Some kits shown may not be available in all countries.

## English

### System information
**ASTP2:** ACN 20230

### Intended use
In vitro test for the quantitative determination of aspartate aminotransferase (AST) with pyridoxal phosphate activation in human serum and plasma on **cobas c** systems.

### Summary
Aspartate aminotransferase (AST) measurements, performed with this device, in human serum and plasma are used as an aid in diagnosis of hepatocellular injury and in monitoring chronic liver injury.

The enzyme aspartate aminotransferase (AST) is widely distributed in tissue, primarily in the liver, cardiac muscle, skeletal muscle, kidney, brain and erythrocytes.[1] AST catalyzes the transfer of amino groups from L-aspartate to α-ketoglutarate, resulting in L-glutamate and oxaloacetate. This is a critical process of the tricarboxylic acid cycle, in which the coenzyme pyridoxal phosphate (also known as pyridoxal-5-phosphate or active vitamin B6) is required. In particular, AST is vital for aerobic glycolysis. AST exists in human tissues as 2 distinct isoenzymes, 1 located in the cytoplasm (c-AST), and the other in mitochondria (m-AST), which differ in amino acid composition and immunochemical and kinetic properties. In healthy individuals, the circulating AST levels consist mainly of cytoplasmic AST, originating from cytoplasmic leakage, on the other side, mitochondrial AST activity in serum shows a marked increase in patients with extensive liver cell degeneration and necrosis. Although AST activity is important in all cells with high metabolic activity, it is more relevant for liver and muscle cells.[2]

Primarily, AST is a marker of hepatocellular injury. Measurement of AST activity is therefore used for the diagnosis of hepatic diseases such as acute and chronic viral hepatitis, nonalcoholic fatty liver disease (NAFLD), alcohol-related liver disease, ischemic hepatopathy, suspected malignant infiltration, cholestasis.[3] Although alanine aminotransferase (ALT) is considered a more specific indicator of liver disease, the concentration of AST may be a more sensitive indicator of liver injury in conditions such as alcohol-related liver disease and in some cases of autoimmune hepatitis.[4] Several international guidelines recommend AST testing for monitoring chronic hepatitis status and progression.[4,5]

Non-liver causes for increases in AST include damage to cardiac or skeletal muscle cells and haemolysis. Serum elevation of AST without elevation in ALT is suggestive of cardiac or muscle disease.[3] In patients undergoing renal dialysis or those with vitamin B6 deficiency, serum AST may be decreased.[6] AST serum levels can be affected by age, gender, alcohol consumption, body mass index, dietary and living habits, nutrition, metabolic status, and drug treatment, among other factors.[7]

In patients with vitamin B6 deficiency (insufficient endogenous pyridoxal phosphate), serum aminotransferase activity may be decreased. The addition of pyridoxal phosphate to this assay causes an increase in aminotransferase activity (activation higher for AST than for ALT) and prevents falsely low aminotransferase test results in these samples.[1]

### Test principle
This assay follows the recommendations of the IFCC, but was optimized for performance and stability.[8]

AST catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. The oxaloacetate then reacts with NADH, in the presence of malate dehydrogenase (MDH), to form L-malate and NAD$^+$. Pyridoxal phosphate serves as a coenzyme in the amino transfer reaction. It ensures full enzyme activation.

$$\text{L-Aspartate + 2-oxoglutarate} \xrightarrow{\text{AST}} \text{oxaloacetate + L-glutamate}$$

$$\text{Oxaloacetate + NADH + H}^+ \xrightarrow{\text{MDH}} \text{L-malate + NAD}^+$$

The rate of the NADH oxidation is directly proportional to the catalytic AST activity. It is determined by measuring the decrease in absorbance.

### Reagents - working solutions

**R1** TRIS buffer: 180 mmol/L, pH 7.65 (37 °C); L-aspartate: 550 mmol/L; MDH (microorganisms): ≥ 11 µkat/L; LDH (microorganisms): ≥ 80 µkat/L; pyridoxamine phosphate: 0.23 mmol/L; albumin (bovine): 0.25 %; stabilizers; preservative

**R3** NADH: ≥ 0.71 mmol/L; 2-oxoglutarate: 96 mmol/L; preservative

R1 is in position B and R3 is in position C.

### Precautions and warnings
For in vitro diagnostic use for laboratory professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

### Reagent handling
Ready for use

### Storage and stability

| | |
|---|---|
| Shelf life at 2-8 °C: | See expiration date on **cobas c** pack label. |
| On-board in use and refrigerated on the analyzer: | 12 weeks |

### Specimen collection and preparation
For specimen collection and preparation only use suitable tubes or collection containers.

# ASTP2

**Aspartate Aminotransferase acc. to IFCC II**

Only the specimens listed below were tested and found acceptable.
Serum
Plasma: Li-heparin and $K_2$- and $K_3$-EDTA plasma

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

See the limitations and interferences section for details about possible sample interferences.

| Stability: | 4 days at 15-25 °C |
|---|---|
| | 7 days at 2-8 °C |
| | 3 months at -20 °C (± 5 °C) |

Freeze only once.

## Materials provided

See "Reagents – working solutions" section for reagents.

## Materials required (but not provided)

See "Order information" section
General laboratory equipment

## Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

## Application for serum and plasma

### Test definition

| Reaction time | 10 min |
|---|---|
| Wavelength (sub/main) | 700/340 nm |

| Reagent pipetting | | Diluent ($H_2O$) |
|---|---|---|
| R1 | 52 µL | 48 µL |
| R3 | 15 µL | – |

| Sample volumes | Sample | Sample dilution | |
|---|---|---|---|
| | | Sample | Diluent (NaCl) |
| Normal | 4.5 µL | – | – |
| Decreased | 4.5 µL | 10 µL | 90 µL |
| Increased | 4.5 µL | – | – |

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

## Calibration

| Calibrators | S1: $H_2O$ |
|---|---|
| | S2: C.f.a.s. |
| Calibration mode | Linear |
| Calibration frequency | Automatic full calibration |
| | - after reagent lot change |
| | Full calibration |
| | - as required following quality control procedures |

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against the original IFCC formulation using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ε.[8]

## Quality control

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. It is recommended to perform quality control always after lot calibration and subsequently at least every 12 weeks. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

## Calculation

**cobas c** systems automatically calculate the analyte activity of each sample in the unit U/L (µkat/L).

Conversion factor: U/L x 0.0167 = µkat/L

## Limitations - interference

Criterion: Recovery within ± 4.0 U/L of initial values of samples ≤ 40 U/L and ± 10 % for samples > 40 U/L.

Icterus:[9] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[9] No significant interference up to an H index of 25 (approximate hemoglobin concentration: 15.6 µmol/L or 25 mg/dL).
Contamination with erythrocytes will elevate results, because the analyte level in erythrocytes is higher than in normal sera. The level of interference may be variable depending on the content of analyte in the lysed erythrocytes.

Lipemia (Intralipid):[9] No significant interference up to an L index of 500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Lipemic specimens may cause > Abs flagging.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[10,11]

Drug interferences are measured based on recommendations given in CLSI guidelines EP07 and EP37 and other published literature. Effects of concentrations exceeding these recommendations have not been characterized.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[12]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

## ACTION REQUIRED

**Special Wash Programming:** The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

## Limits and ranges

### Measuring range

5-700 U/L (0.08-11.7 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 10.

### Lower limits of measurement

*Limit of Blank, Limit of Detection and Limit of Quantitation*

| Limit of Blank | = 5 U/L (0.08 µkat/L) |
|---|---|
| Limit of Detection | = 5 U/L (0.08 µkat/L) |
| Limit of Quantitation | = 5 U/L (0.08 µkat/L) |

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

# ASTP2

**Aspartate Aminotransferase acc. to IFCC II**

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the activity below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low activity samples.

The Limit of Detection corresponds to the lowest analyte activity which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte activity that can be reproducibly measured with a total error of 20 %. It has been determined using low activity aspartate aminotransferase samples.

## Expected values

### U/L

Acc. to IFCC/Standard Method 94 with pyridoxal phosphate activation measured at 37 °C:[13]

| Males: | 10-50 U/L |
|---|---|
| Females: | 10-35 U/L |

Consensus values with pyridoxal phosphate activation:[14]

| Males: | up to 50 U/L |
|---|---|
| Females: | up to 35 U/L |

### µkat/L*

Acc. to IFCC/Standard Method 94 with pyridoxal phosphate activation measured at 37 °C:[13]

| Males: | 0.17-0.84 µkat/L |
|---|---|
| Females: | 0.17-0.58 µkat/L |

Consensus values with pyridoxal phosphate activation:[14]

| Males: | up to 0.84 µkat/L |
|---|---|
| Females: | up to 0.58 µkat/L |

*calculated by unit conversion factor

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

## Specific performance data

Representative performance data on the analyzers are given below. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

## Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

| Repeatability | Mean U/L | SD U/L | CV % |
|---|---|---|---|
| PCCC1[a] | 46.4 | 0.551 | 1.2 |
| PCCC2[b] | 149 | 1.49 | 1.0 |
| Human serum 1 | 11.3 | 0.242 | 2.1 |
| Human serum 2 | 33.0 | 0.497 | 1.5 |
| Human serum 3 | 50.4 | 0.314 | 0.6 |
| Human serum 4 | 345 | 1.31 | 0.4 |
| Human serum 5 | 651 | 2.19 | 0.3 |

| Intermediate precision | Mean U/L | SD U/L | CV % |
|---|---|---|---|
| PCCC1[a] | 46.5 | 1.33 | 2.9 |
| PCCC2[b] | 148 | 3.30 | 2.2 |
| Human serum 1 | 11.4 | 0.281 | 2.5 |
| Human serum 2 | 33.0 | 0.552 | 1.7 |
| Human serum 3 | 50.4 | 0.371 | 0.7 |
| Human serum 4 | 345 | 1.79 | 0.5 |
| Human serum 5 | 651 | 3.63 | 0.6 |

a) PreciControl ClinChem Multi 1
b) PreciControl ClinChem Multi 2

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s) and **cobas c** 703 analyzer(s).

## Method comparison

AST values for human serum and plasma samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using the test ASTLP on a **cobas c** 501 analyzer (x).

Sample size (n) = 111

| Passing/Bablok[15] | Linear regression |
|---|---|
| y = 0.960x + 2.35 U/L | y = 0.931x + 6.22 U/L |
| τ = 0.978 | r = 0.998 |

The sample activities were between 7.50 and 694 U/L.

AST values for human serum and plasma samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

Sample size (n) = 50

| Passing/Bablok[15] | Linear regression |
|---|---|
| y = 0.984x + 0.903 U/L | y = 0.980x + 1.37 U/L |
| τ = 0.989 | r = 1.000 |

The sample activities were between 7.79 and 667 U/L.

AST values for human serum and plasma samples obtained on a **cobas c** 703 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

Sample size (n) = 65

| Passing/Bablok[15] | Linear regression |
|---|---|
| y = 0.991x + 0.793 U/L | y = 0.989x + 1.15 U/L |
| τ = 0.981 | r = 1.000 |

The sample concentrations were between 6.29 and 667 U/L.

## References

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

## Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

| | |
|---|---|
| CONTENT | Contents of kit |
| ⟶ | Volume for reconstitution |
| GTIN | Global Trade Item Number |
| Rx only | For USA: Caution: Federal law restricts this device to sale by or on the order of a physician. |

CE 0123

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Roche

# BILD2

**Bilirubin Direct Gen.2**

**cobas®**

## Order information

| REF | 📖 i | CONTENT | | Analyzer(s) on which **cobas c** pack(s) can be used |
|---|---|---|---|---|
| 08056951190 | 08056951500 | Bilirubin Direct Gen.2 (1000 tests) | System-ID 2030 001 | **cobas c** 303, **cobas c** 503 |

Materials required (but not provided):

| | | |
|---|---|---|
| 10759350190 | Calibrator f.a.s. (12 × 3 mL) | Code 20401 |
| 05117003190 | PreciControl ClinChem Multi 1 (20 x 5 mL) | Code 20391 |
| 05947626190 | PreciControl ClinChem Multi 1 (4 x 5 mL) | Code 20391 |
| 05117216190 | PreciControl ClinChem Multi 2 (20 x 5 mL) | Code 20392 |
| 05947774190 | PreciControl ClinChem Multi 2 (4 x 5 mL) | Code 20392 |
| 10158046122 | Precibil (4 x 2 mL) | Code 20306 |

## English

## System information

*Jendrassik-Grof method*
**BILD2-J:** ACN 20301
*Doumas method*
**BILD2-D:** ACN 20300

## Intended use

In vitro test for the quantitative determination of direct bilirubin in human serum and plasma on **cobas c** systems.

## Summary

Measurements of direct bilirubin, performed with this assay in human serum and plasma of adults and neonates, are used for the diagnosis of hyperbilirubinemia (such as observed with abnormal destruction of red blood cells, liver diseases, and metabolic disorders, including hepatitis and gallbladder block), and in newborn screening for severe hyperbilirubinemia.

Bilirubin is formed in the reticuloendothelial system during the degradation of aged erythrocytes. The heme portion from hemoglobin and from other heme-containing proteins is removed, metabolized to bilirubin, and transported as a complex with serum albumin to the liver. In the liver, bilirubin is conjugated with glucuronic acid for solubilization and subsequent transport through the bile duct and elimination via the digestive tract. Diseases or conditions which, through hemolytic processes, produce bilirubin faster than the liver can metabolize it, cause the levels of unconjugated (indirect) bilirubin to increase in the circulation. Liver immaturity and several other diseases in which the bilirubin conjugation mechanism is impaired cause similar elevations of circulating unconjugated bilirubin. Bile duct obstruction or damage to hepatocellular structure causes increases in the levels of both conjugated (direct) and unconjugated (indirect) bilirubin in the circulation.[1,2,3,4]

In newborns, several mechanisms lead to an increased bilirubin load, such as increased turnover in fetal red blood cells, reduced bilirubin clearance, and increased enterohepatic circulation of bilirubin. Screening neonates for severe hyperbilirubinemia, especially in newborns with infant jaundice, has been proposed to help preventing chronic bilirubin encephalopathy.[5,6]

## Test principle

Diazo method.[7]

Conjugated bilirubin and δ-bilirubin (direct bilirubin) react directly with 3,5 Dichlorophenyl diazonium salt in acid buffer to form the red-colored azobilirubin.

$$\text{bilirubin + 3,5 DPD} \longrightarrow \text{azobilirubin}$$

The color intensity of the red azo dye formed is directly proportional to the direct (conjugated) bilirubin concentration and can be determined photometrically.

Remark: Under the influence of blue light, e.g. during phototherapy of newborn children, unconjugated bilirubin is partly transformed into a water-soluble isomer called photobilirubin, a substrate for direct bilirubin tests. This fraction is detected by BILD2 and may lead to above-normal results in healthy children.

## Reagents - working solutions

**R1** Phosphoric acid: 85 mmol/L; HEDTA: 4.0 mmol/L; NaCl: 50 mmol/L; detergent; pH 1.9

**R2** 3,5-Dichlorophenyl diazonium: 1.5 mmol/L; pH 1.3

R1 is in position B and R2 is in position C.

## Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

## Reagent handling

Ready for use

## Storage and stability

| | |
|---|---|
| Shelf life at 2-8 °C: | See expiration date on **cobas c** pack label. |
| On-board in use and refrigerated on the analyzer: | 26 weeks |

## Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Serum: Collect serum using standard sample tubes.
Plasma: Li-heparin, $K_2$-, $K_3$-EDTA.

Protect specimens from exposure to light.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

See the limitations and interferences section for details about possible sample interferences.

| Stability:[a),8,9] | 2 days at 15-25 °C |
|---|---|
| | 7 days at 2-8 °C |
| | 6 months at (-15)-(-25) °C |

a) If care is taken to prevent exposure to light
Freeze only once.

## Materials provided

See "Reagents – working solutions" section for reagents.

# BILD2
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## Materials required (but not provided)
See "Order information" section

General laboratory equipment

## Assay
For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

## Application for serum and plasma

### Test definition

| | | |
|---|---|---|
| Reporting time | 10 min | |
| Wavelength (sub/main) | 800/546 nm | |
| Reagent pipetting | | Diluent (NaCl) |
| R1 | 79 µL | – |
| R2 | 16 µL | – |

| Sample volumes | Sample | Sample dilution | |
|---|---|---|---|
| | | Sample | Diluent ($H_2O$) |
| Normal | 4.4 µL | – | – |
| Decreased | 2.2 µL | – | – |
| Increased | 4.4 µL | – | – |

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

### Calibration

| | |
|---|---|
| Calibrator | S1: $H_2O$ |
| | S2: C.f.a.s. |
| Calibration mode | Linear regression |
| Calibration frequency | Automatic full calibration<br>- after reagent lot change |
| | Full calibration<br>- as required following quality control procedures |

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against the manual test performance using the Jendrassik-Grof or Doumas method.[10,11]

### Quality control
For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. It is recommended to perform quality control always after lot calibration and subsequently at least every 26 weeks.

Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

### Calculation
**cobas c** systems automatically calculate the analyte concentration of each sample in the unit µmol/L (mg/dL, mg/L).

| Conversion factors: | µmol/L x 0.0585 = mg/dL |
|---|---|
| | µmol/L x 0.585 = mg/L |

## Limitations - interference
Criterion: Recovery within ± 10 % of initial values at a direct bilirubin concentration of 34.2 µmol/L (2.0 mg/dL).

Hemolysis:[12] No significant interference up to an H index of 25 (approximate hemoglobin concentration: 15.5 µmol/L or 25 mg/dL).

Lipemia (Intralipid):[12] No significant interference up to an L index of 750. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[13,14]

Exception: Phenylbutazone causes artificially low bilirubin results.

Samples containing indocyanine green must not be measured.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[15]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

In certain cases specimens may give a direct bilirubin result slightly greater than the total bilirubin result. This is observed in patient samples when nearly all the reacting bilirubin is in the direct form. In such cases the result for the total bilirubin should be reported for both direct bilirubin and total bilirubin values.

### ACTION REQUIRED
**Special Wash Programming:** The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

### Limits and ranges
#### Measuring range
*Jendrassik-Grof method*

1.5-291 µmol/L (0.09-17 mg/dL)

*Doumas method*

1.4-236 µmol/L (0.08-14 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.

#### Lower limits of measurement
*Jendrassik-Grof method*

*Limit of Blank, Limit of Detection and Limit of Quantitation*

| | |
|---|---|
| Limit of Blank | = 1.0 µmol/L (0.06 mg/dL) |
| Limit of Detection | = 1.5 µmol/L (0.09 mg/dL) |
| Limit of Quantitation | = 3.0 µmol/L (0.18 mg/dL) |

The Limit of Blank, the Limit of Detection and the Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a total error of 30 %. It has been determined using low concentration bilirubin samples.

#### Lower limits of measurement
*Doumas method*

*Limit of Blank, Limit of Detection and Limit of Quantitation*

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| | | |
|---|---|---|
| Limit of Blank | = 0.8 µmol/L (0.05 mg/dL) | |
| Limit of Detection | = 1.2 µmol/L (0.07 mg/dL) | |
| Limit of Quantitation | = 1.4 µmol/L (0.08 mg/dL) | |

The Limit of Blank, the Limit of Detection and the Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a total error of 30 %. It has been determined using low concentration bilirubin samples.

## Expected values

*Jendrassik-Grof method*[1]

**µmol/L**

Direct bilirubin ≤ 5 µmol/L

**mg/dL**

Direct bilirubin ≤ 0.30 mg/dL

An upper limit of 10 µmol/L direct bilirubin for neonates has been cited in the literature, although this has not been confirmed by internal data.[16]

*Doumas method*[17]

**µmol/L**

Direct bilirubin ≤ 3.4 µmol/L

**mg/dL**

Direct bilirubin ≤ 0.20 mg/dL

An upper limit of 10 µmol/L direct bilirubin for neonates has been cited in the literature, although this has not been confirmed by internal data.[16]

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

## Specific performance data

Representative performance data on the analyzers are given below. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

## Precision

*Jendrassik-Grof method*

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

| Repeatability | Mean µmol/L | SD µmol/L | CV % |
|---|---|---|---|
| PCCC1[b] | 16.5 | 0.116 | 0.7 |
| PCCC2[c] | 44.1 | 0.216 | 0.5 |
| Human serum 1 | 4.32 | 0.0810 | 1.9 |
| Human serum 2 | 9.76 | 0.141 | 1.4 |
| Human serum 3 | 89.8 | 0.203 | 0.2 |
| Human serum 4 | 139 | 0.488 | 0.4 |
| Human serum 5 | 254 | 0.756 | 0.3 |

| Intermediate precision | Mean µmol/L | SD µmol/L | CV % |
|---|---|---|---|
| PCCC1[b] | 16.5 | 0.212 | 1.3 |
| PCCC2[c] | 44.1 | 0.573 | 1.3 |
| Human serum 1 | 4.31 | 0.107 | 2.5 |
| Human serum 2 | 9.76 | 0.241 | 2.5 |
| Human serum 3 | 89.8 | 0.615 | 0.7 |
| Human serum 4 | 139 | 2.23 | 1.6 |
| Human serum 5 | 254 | 2.42 | 1.0 |

b) PreciControl ClinChem Multi 1
c) PreciControl ClinChem Multi 2

*Doumas method*

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

| Repeatability | Mean µmol/L | SD µmol/L | CV % |
|---|---|---|---|
| PCCC1[b] | 13.2 | 0.0838 | 0.6 |
| PCCC2[c] | 35.6 | 0.146 | 0.4 |
| Human serum 1 | 3.23 | 0.0673 | 2.1 |
| Human serum 2 | 8.64 | 0.0899 | 1.0 |
| Human serum 3 | 57.2 | 0.179 | 0.3 |
| Human serum 4 | 109 | 0.393 | 0.4 |
| Human serum 5 | 195 | 0.512 | 0.3 |

| Intermediate precision | Mean µmol/L | SD µmol/L | CV % |
|---|---|---|---|
| PCCC1[b] | 13.2 | 0.175 | 1.3 |
| PCCC2[c] | 35.9 | 0.429 | 1.2 |
| Human serum 1 | 3.32 | 0.0945 | 2.8 |
| Human serum 2 | 8.64 | 0.176 | 2.0 |
| Human serum 3 | 57.8 | 0.421 | 0.7 |
| Human serum 4 | 110 | 1.89 | 1.7 |
| Human serum 5 | 195 | 1.98 | 1.0 |

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s).

## Method comparison

*Jendrassik-Grof method*

Bilirubin values for human serum and plasma samples obtained with the Roche BILD2 reagent on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 582

| Passing/Bablok[18] | Linear regression |
|---|---|
| y = 1.001x + 0.646 µmol/L | y = 0.987x + 1.28 µmol/L |
| τ = 0.965 | r = 1.000 |

The sample concentrations were between 1.50 and 288 µmol/L.

Bilirubin values for human serum and plasma samples obtained with the Roche BILD2 reagent on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 64

| Passing/Bablok[18] | Linear regression |
|---|---|

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$y = 0.988x + 1.02 \ \mu mol/L$

$\tau = 0.952$

$y = 0.938x + 2.53 \ \mu mol/L$

$r = 0.999$

The sample concentrations were between 1.50 and 276 µmol/L.

*Doumas method*

Bilirubin values for human serum and plasma samples obtained with the Roche BILD2 reagent on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 66

Passing/Bablok[18]

$y = 1.001x + 0.481 \ \mu mol/L$

$\tau = 0.966$

Linear regression

$y = 0.985x + 1.22 \ \mu mol/L$

$r = 0.999$

The sample concentrations were between 1.49 and 231 µmol/L.

Bilirubin values for human serum and plasma samples obtained with the Roche BILD2 reagent on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 62

Passing/Bablok[18]

$y = 0.985x + 0.716 \ \mu mol/L$

$\tau = 0.928$

Linear regression

$y = 0.941x + 1.81 \ \mu mol/L$

$r = 0.999$

The sample concentrations were between 1.40 and 222 µmol/L.

## References

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13  Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.

14  Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.

15  Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

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18  Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

## Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see navifyportal.roche.com for definition of symbols used):

| CONTENT | Contents of kit |
| --- | --- |
| → | Volume for reconstitution |
| GTIN | Global Trade Item Number |
| Rx only | For USA: Caution: Federal law restricts this device to sale by or on the order of a physician. |

CE 0123

Roche

# BILT3

**Bilirubin Total Gen.3**

Order information

| REF | 🏷i | CONTENT | | Analyzer(s) on which **cobas c** pack(s) can be used |
|---|---|---|---|---|
| 08056960190 | 08056960500 | Bilirubin Total Gen.3 (1050 tests) | System-ID 2031 001 | **cobas c** 303, **cobas c** 503 |

Materials required (but not provided):

| | | |
|---|---|---|
| 10759350190 | Calibrator f.a.s. (12 x 3 mL) | Code 20401 |
| 10158046122 | Precibil (4 x 2 mL) | Code 20306 |
| 05117003190 | PreciControl ClinChem Multi 1 (20 x 5 mL) | Code 20391 |
| 05947626190 | PreciControl ClinChem Multi 1 (4 x 5 mL) | Code 20391 |
| 05117216190 | PreciControl ClinChem Multi 2 (20 x 5 mL) | Code 20392 |
| 05947774190 | PreciControl ClinChem Multi 2 (4 x 5 mL) | Code 20392 |
| 08063494190 | Diluent NaCl 9 % (123 mL) | System-ID 2906 001 |

## English

### System information

**BILT3:** ACN 20310

### Intended use

In vitro test for the quantitative determination of total bilirubin in serum and plasma of adults and neonates on **cobas c** systems.

### Summary

Measurements of total bilirubin, performed with this assay in human serum and plasma of adults and neonates, are used for the diagnosis of hyperbilirubinemia (such as observed with abnormal destruction of red blood cells, liver diseases, and metabolic disorders, including hepatitis and gallbladder block), and in newborn screening for severe hyperbilirubinemia. Total bilirubin is a combination of direct and indirect bilirubin.

Bilirubin is formed in the reticuloendothelial system during the degradation of aged erythrocytes. The heme portion from hemoglobin and from other heme-containing proteins is removed, metabolized to bilirubin, and transported as a complex with serum albumin to the liver. In the liver, bilirubin is conjugated with glucuronic acid for solubilization and subsequent transport through the bile duct and elimination via the digestive tract.

Diseases or conditions which, through hemolytic processes, produce bilirubin faster than the liver can metabolize it, cause the levels of unconjugated (indirect) bilirubin to increase in the circulation. Liver immaturity and several other diseases in which the bilirubin conjugation mechanism is impaired cause similar elevations of circulating unconjugated bilirubin. Bile duct obstruction or damage to hepatocellular structure causes increases in the levels of both conjugated (direct) and unconjugated (indirect) bilirubin in the circulation.[1,2,3,4]

Numerous guidelines, including those from the World Health Organization, the American College of Gastroenterology, and National Institute for Health and Care Excellence, recommend bilirubin testing as part of the diagnostic workup for liver injury.[3,4,5,6,7]

In newborns, several mechanisms lead to an increased bilirubin load, such as increased turnover in fetal red blood cells, reduced bilirubin clearance, and increased enterohepatic circulation of bilirubin. Screening neonates for severe hyperbilirubinemia, especially in newborns with infant jaundice, has been proposed to help preventing chronic bilirubin encephalopathy.[8,9] For infants born at >= 35 weeks of gestation, the American Academy of Pediatrics Subcommittee on Hyperbilirubinemia recommends to measure total serum bilirubin in case of jaundice in the first 24 hours after birth or if jaundice appears excessive for infants' age (all bilirubin levels should be interpreted according to the infant's age in hours).[8]

### Test principle[10]

Colorimetric diazo method

Total bilirubin, in the presence of a suitable solubilizing agent, is coupled with 3,5-dichlorophenyl diazonium in a strongly acidic medium.

$$\text{Bilirubin} + \text{3,5-DPD} \xrightarrow{\text{acid}} \text{azobilirubin}$$

The color intensity of the red azo dye formed is directly proportional to the total bilirubin and can be determined photometrically.

### Reagents - working solutions

| | |
|---|---|
| **R1** | Phosphate: 50 mmol/L; detergents; stabilizers; pH 1.0 |
| **R3** | 3,5-dichlorophenyl diazonium salt: ≥ 1.35 mmol/L |

R1 is in position B and R3 is in position C.

### Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Danger

| | |
|---|---|
| H290 | May be corrosive to metals. |
| H319 | Causes serious eye irritation. |
| H360FD | May damage fertility. May damage the unborn child. |

**Prevention:**

| | |
|---|---|
| P201 | Obtain special instructions before use. |
| P280 | Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection. |

**Response:**

| | |
|---|---|
| P308 + P313 | IF exposed or concerned: Get medical advice/attention. |
| P337 + P313 | If eye irritation persists: Get medical advice/attention. |
| P390 | Absorb spillage to prevent material damage. |

**Disposal:**

| | |
|---|---|
| P501 | Dispose of contents/container to an approved waste disposal plant. |

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

### Reagent handling

Ready for use

# BILT3

**Bilirubin Total Gen.3**

cobas®

## Storage and stability

| | |
|---|---|
| Shelf life at 2-8 °C: | See expiration date on **cobas c** pack label. |
| On-board in use and refrigerated on the analyzer: | 6 weeks |

## Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Serum
Plasma: Li-heparin and $K_2$-, $K_3$-EDTA plasma

(The use of EDTA-plasma with elevated hematocrit may lead to slightly lower values.)

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

See the limitations and interferences section for details about possible sample interferences.

| Stability:[a],[11] | 1 day at 15-25 °C |
|---|---|
| | 7 days at 2-8 °C |
| | 6 months at (-15)-(-25) °C |

a) If care is taken to prevent exposure to light

Freeze only once.

## Materials provided

See "Reagents – working solutions" section for reagents.

## Materials required (but not provided)

See "Order information" section
General laboratory equipment

## Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

## Application for serum and plasma

### Test definition

| Reporting time | 10 min | | |
|---|---|---|---|
| Wavelength (sub/main) | 600/546 nm | | |
| Reagent pipetting | Diluent ($H_2O$) | | |
| R1 | 78 µL | – | |
| R3 | 16 µL | – | |

| Sample volumes | Sample | Sample dilution | |
|---|---|---|---|
| | | Sample | Diluent (NaCl) |
| Normal | 1.3 µL | – | – |
| Decreased | 2.6 µL | 20 µL | 60 µL |
| Increased | 1.3 µL | – | – |

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

## Calibration

| Calibrators | S1: $H_2O$ |
|---|---|
| | S2: C.f.a.s. |
| Calibration mode | Linear |
| Calibration frequency | Automatic full calibration |
| | - after reagent lot change |
| | Full calibration |
| | - as required following quality control procedures |

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: The method was standardized against the Doumas method.[12]

## Quality control

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. It is recommended to perform quality control always after lot calibration and subsequently at least every 6 weeks.

Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

## Calculation

**cobas c** systems automatically calculate the analyte concentration of each sample in the unit µmol/L (mg/dL, mg/L).

| Conversion factors: | µmol/L x 0.0585 = mg/dL |
|---|---|
| | µmol/L x 0.585 = mg/L |

## Limitations - interference

Criterion: Recovery within ± 3.4 µmol/L (0.199 mg/dL) of initial values of samples ≤ 34 µmol/L (1.99 mg/dL) and within ± 10 % for samples > 34 µmol/L.

Hemolysis:[13] No significant interference up to an H index of 800 (approximate hemoglobin concentration: 497 µmol/L or 800 mg/dL).

Immunoglobulins: No significant interference from immunoglobulins up to a concentration of 28 g/L (187 µmol/L) (simulated by human immunoglobulin G).

Criterion: Recovery within ± 1.7 µmol/L (0.099 mg/dL) of initial values of samples ≤ 17 µmol/L (0.995 mg/dL) and within ± 10 % for samples > 17 µmol/L.

Hemolysis in neonates:[13] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[13] No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[14,15]

Indican: No significant interference from indican up to a concentration of 0.12 mmol/L (3 mg/dL).

Cyanokit (Hydroxocobalamin) may cause falsely low results.

Samples containing indocyanine green must not be measured.

Results from certain multiple myeloma patients may show a positive bias in recovery. Not all multiple myeloma patients show the bias and the severity of the bias may vary between patients.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[16]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

In certain cases specimens may give a direct bilirubin result slightly greater than the total bilirubin result. This is observed in patient samples when nearly all the reacting bilirubin is in the direct form. In such cases the result

# BILT3

**Bilirubin Total Gen.3**

<div style="text-align: right;">

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</div>

for the total bilirubin should be reported for both D-bilirubin and total bilirubin values.

## ACTION REQUIRED

**Special Wash Programming:** The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions refer to the operator's manual.

## Limits and ranges

### Measuring range

2.5-650 µmol/L (0.146-38.0 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.

### Lower limits of measurement

*Limit of Blank, Limit of Detection and Limit of Quantitation*

| | |
|---|---|
| Limit of Blank | = 1.7 µmol/L (0.099 mg/dL) |
| Limit of Detection | = 2.5 µmol/L (0.146 mg/dL) |
| Limit of Quantitation | = 2.5 µmol/L (0.146 mg/dL) |

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a total error of 30 %. It has been determined using low concentration bilirubin samples.

## Expected values

### µmol/L

| | |
|---|---|
| Adults[17] | up to 21 µmol/L |
| Children with age ≥ 1 month[17] | up to 17 µmol/L |

Reference range study with 500 well-characterized human serum samples:[18]

| | |
|---|---|
| Males | up to 24 µmol/L |
| Females | up to 15 µmol/L |

High risk for developing clinically significant hyperbilirubinemia:

Newborns: Term and near-term[19]

Age of newborn:

| | |
|---|---|
| 24 hours | ≥ 137 µmol/L[b] |
| 48 hours | ≥ 222 µmol/L[b] |
| 84 hours | ≥ 290 µmol/L[b] |

b) 95th percentile

Levels > 95th percentile: Such levels of hyperbilirubinemia have been deemed significant and are generally considered to require close supervision, possible further evaluation, and sometimes intervention.

### mg/dL

| | |
|---|---|
| Adults[17] | up to 1.2 mg/dL |
| Children with age ≥ 1 month[17] | up to 1.0 mg/dL |

Reference range study with 500 well-characterized human serum samples:[18]

| | |
|---|---|
| Males | up to 1.4 mg/dL |
| Females | up to 0.9 mg/dL |

High risk for developing clinically significant hyperbilirubinemia:

Newborns: Term and near-term[19]

Age of newborn:

| | |
|---|---|
| 24 hours | ≥ 8.0 mg/dL[b] |
| 48 hours | ≥ 13.0 mg/dL[b] |
| 84 hours | ≥ 17.0 mg/dL[b] |

b) 95th percentile

Levels > 95th percentile: Such levels of hyperbilirubinemia have been deemed significant and are generally considered to require close supervision, possible further evaluation, and sometimes intervention.

**Nomogram for designation of risk in 2840 well newborns**[19]

Serum bilirubin



Postnatal age (hours)
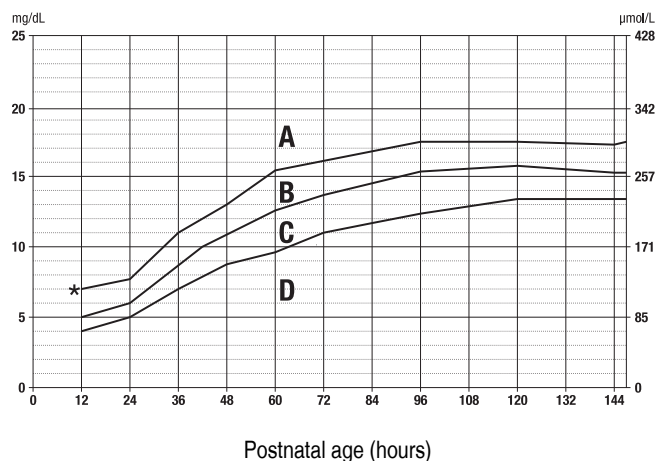
* 95th percentile

| | |
|---|---|
| **A** High risk zone | **C** Low intermediate risk zone |
| **B** High intermediate risk zone | **D** Low risk zone |

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

### Specific performance data

Representative performance data on the analyzers are given below. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

### Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

| *Repeatability* | *Mean* | *SD* | *CV* |
|---|---|---|---|
| | *µmol/L* | *µmol/L* | *%* |
| PCCC1[c] | 16.2 | 0.256 | 1.6 |
| PCCC2[d] | 61.4 | 0.315 | 0.5 |
| Human serum 1 | 5.43 | 0.211 | 3.9 |
| Human serum 2 | 21.5 | 0.228 | 1.1 |

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| | | | |
|---|---|---|---|
| Human serum 3 | 91.6 | 0.507 | 0.6 |
| Human serum 4 | 295 | 1.24 | 0.4 |
| Human serum 5 | 519 | 1.97 | 0.4 |

| Intermediate precision | Mean | SD | CV |
|---|---|---|---|
| | µmol/L | µmol/L | % |
| PCCC1[c] | 16.2 | 0.372 | 2.3 |
| PCCC2[d] | 60.9 | 0.630 | 1.0 |
| Human serum 1 | 5.43 | 0.222 | 4.1 |
| Human serum 2 | 21.4 | 0.269 | 1.3 |
| Human serum 3 | 91.6 | 0.706 | 0.8 |
| Human serum 4 | 295 | 1.57 | 0.5 |
| Human serum 5 | 516 | 3.26 | 0.6 |

c) PreciControl ClinChem Multi 1

d) PreciControl ClinChem Multi 2

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s).

## Method comparison

Total bilirubin values for human serum and plasma samples obtained with the Roche Bilirubin Total Gen.3 reagent on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 649

| Passing/Bablok[20] | Linear regression |
|---|---|
| y = 1.000x - 0.0394 µmol/L | y = 1.002x - 0.339 µmol/L |
| τ = 0.979 | r = 1.000 |

The sample concentrations were between 2.51 and 622 µmol/L.

Total bilirubin values for human serum and plasma samples obtained with the Roche Bilirubin Total Gen.3 reagent on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 67

| Passing/Bablok[20] | Linear regression |
|---|---|
| y = 1.010x - 0.247 µmol/L | y = 1.008x - 0.264 µmol/L |
| τ = 0.966 | r = 1.000 |

The sample concentrations were between 2.90 and 615 µmol/L.

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16  Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

## Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see navifyportal.roche.com for definition of symbols used):

| | |
|---|---|
| CONTENT | Contents of kit |
| ⟶ | Volume for reconstitution |
| GTIN | Global Trade Item Number |
| Rx only | For USA: Caution: Federal law restricts this device to sale by or on the order of a physician. |

# BILT3

**Bilirubin Total Gen.3**

$CE$ 0123

Roche

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# C.f.a.s. HbA1c

**cobas**®

REF 04528417190    i 04528417500    → 3 x 2 mL Calibrator

## English

### System information

For use on **cobas c** and COBAS INTEGRA analyzer systems, refer to the corresponding method sheet of the assay for the identification on the systems.

### Intended use

C.f.a.s. (Calibrator for automated systems) HbA1c is for use in the calibration of quantitative Roche methods on Roche clinical chemistry analyzers as specified in the enclosed value sheets.

### Summary

C.f.a.s. HbA1c is a lyophilized calibrator based on hemolyzed sheep blood. The concentrations of the calibrator components have been adjusted to ensure optimal calibration of the appropriate Roche methods on clinical chemistry analyzers.

Some methods specified in the relevant value sheet may not be available in all countries.

### Reagents - working solutions

*Reactive components:*

Hemolyzed sheep blood with chemical additives and material of biological origin as specified. The origin of the biological additives is as follows:

| Analyte | Origin |
|---------|--------|
| Hemoglobin | Sheep blood |
| HbA1c | Human blood |

*Non-reactive components:*

Preservatives and stabilizers

The concentrations of the calibrator components are lot-specific. The exact calibrator values are given in the electronically available or enclosed value sheets.

For Tina-quant Hemoglobin A1cDx Gen.3 on the **cobas c** 513 analyzer and on the **cobas c** 303/503 analyzer, C.f.a.s. HbA1c calibrator values are reagent lot matched. For each application and each combination of C.f.a.s. HbA1c calibrator lot and Tina-quant Hemoglobin A1cDx Gen.3 reagent lot, the exact calibrator values are given in the electronically available or enclosed value sheet.

For all other systems (**cobas c** 111, COBAS INTEGRA 400 plus analyzer, **cobas c** 311/501 and **cobas c** 502 analyzers) for Tina-quant Hemoglobin A1c Gen.3, C.f.a.s. HbA1c calibrator values are not reagent lot matched.

For the **cobas c** analyzers (except for the **cobas c** 111 analyzer) the values are encoded in electronic files sent via the **cobas** link to the analyzers.

### Calibrator values

The calibrator values were determined using the method stated in the electronically available or enclosed value sheets. Determinations were performed under strictly standardized conditions on Roche analyzers using Roche system reagents and calibrators according to the standardization plan of the respective assay.

The calibrator values were obtained via single determinations performed in different laboratories, in several separate runs. The calibrator value specified is the mean of all values obtained.

Traceability information is given in the relevant Method Sheets for the system reagent.

### Precautions and warnings

For in vitro diagnostic use for laboratory professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

All human material should be considered potentially infectious. All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods use assays that have been approved or cleared by the FDA or that are in compliance with the legal rules of the European Union (IVDR 2017/746/EU, IVDD 98/79/EC, Annex II, List A). However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[1,2]

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Danger

| H317 | May cause an allergic skin reaction. |
|------|--------------------------------------|
| H318 | Causes serious eye damage. |
| H410 | Very toxic to aquatic life with long lasting effects. |

**Prevention:**

| P261 | Avoid breathing dust. |
|------|------------------------|
| P273 | Avoid release to the environment. |
| P280 | Wear protective gloves/ eye protection/ face protection. |

**Response:**

| P305 + P351 + P338 + P310 | IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor. |
|------|------|
| P333 + P313 | If skin irritation or rash occurs: Get medical advice/attention. |
| P391 | Collect spillage. |

**Hazardous components:**

- tetradonium bromide
- 2-methyl-2H-isothiazol-3-one hydrochloride

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

### Handling

Carefully open one bottle, avoiding the loss of lyophilizate, and pipette in exactly 2.0 mL of distilled/deionized water. Carefully close the bottle and dissolve the contents completely by occasional gentle swirling within 30 minutes. Avoid the formation of foam.

The enclosed barcoded labels are intended exclusively for **cobas c** systems (except for the **cobas c** 513 analyzer) to identify the calibrator. Attach the barcoded labels to the tubes carrying the sample cups containing the calibrator material.

### Storage and stability

Store at 2-8 °C.

Criterion for the stability data stated by Roche:

Recovery within ± 10 % of initial value.

Stability of the lyophilized calibrator at 2-8 °C:

up to the stated expiration date.

Stability of the components in the reconstituted calibrator:

| at 15-25 °C | 8 hours |
|-------------|---------|

# C.f.a.s. HbA1c

**cobas®**

| | |
|---|---|
| at 2-8 °C | 2 days |
| at (−15)-(−25) °C | 3 months (when frozen once) |

Store calibrator tightly capped when not in use.

## Materials provided

See "Reagents – working solutions" section for reagents.

Barcoded labels

## Materials required (but not provided)

Roche system reagents and clinical chemistry analyzers
General laboratory equipment

## Assay

Use C.f.a.s. HbA1c as specified in the relevant Method Sheet for the system reagents.

## References

1  Occupational Safety and Health Standards: Bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.

2  Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

The Summary of Safety & Performance Report can be found here: https://ec.europa.eu/tools/eudamed

## Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

| | |
|---|---|
| CONTENT | Contents of kit |
| CALIBRATOR | Calibrator |
| → | Volume for reconstitution |
| GTIN | Global Trade Item Number |
| Rx only | For USA: Caution: Federal law restricts this device to sale by or on the order of a physician. |

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All other product names and trademarks are the property of their respective owners.

Additions, deletions or changes are indicated by a change bar in the margin.

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CE 0123

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**Roche**

12172623500V9.0

# C.f.a.s. Lipids

**cobas**®

| REF 12172623122      | ☐ᵢ 12172623500      → 3 x 1 mL Calibrator

## English

### System information

For use on **cobas c** and COBAS INTEGRA analyzer systems, refer to the corresponding method sheet of the assay for the identification on the systems.

### Intended use

C.f.a.s. (Calibrator for automated systems) Lipids is for use in the calibration of quantitative Roche methods on Roche clinical chemistry analyzers as specified in the value sheets.

### Summary

C.f.a.s. Lipids is a lyophilized calibrator based on human serum.

The concentrations of the calibrator components have been adjusted to ensure optimal calibration of the appropriate Roche methods on clinical chemistry analyzers.

Some methods specified in the relevant value sheet may not be available in all countries.

### Reagents – working solutions

*Reactive components in the lyophilizate:*

Human serum with chemical additives

*Non-reactive components:*

Preservative and stabilizer

The concentrations of the calibrator components are lot-specific. The exact calibrator values are given in the electronically available or enclosed value sheets.

The values are also encoded in the enclosed calibrator barcode sheets for COBAS INTEGRA and **cobas c** 111 analyzers.

For the **cobas c** analyzers (except for the **cobas c** 111 analyzer) the values are encoded in electronic files sent via the **cobas** link to the analyzers.

### Calibrator values

The calibrator values were determined using the method stated in the electronically available or enclosed value sheets. Determinations were performed under strictly standardized conditions on Roche analyzers using Roche system reagents and the Roche master calibrator.

The calibrator values were obtained via single determinations performed in different laboratories in several separate runs. The calibrator value specified is the mean of all values obtained.

Traceability information is given in the relevant Method Sheets for the system reagents.

### Precautions and warnings

For in vitro diagnostic use for laboratory professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

CAUTION. WARNING: The bottles contain sodium azide (< 1 %). Avoid contact with skin and mucous membranes. Flush affected areas with copious amounts of water. Get immediate medical attention for eyes or if ingested. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up. Clean exposed metal surfaces with 10 % sodium hydroxide.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

H412          Harmful to aquatic life with long lasting effects.

**Prevention:**

P273          Avoid release to the environment.

**Disposal:**

P501          Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

All human material should be considered potentially infectious. All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods use assays that have been approved or cleared by the FDA or that are in compliance with the legal rules of the European Union (IVDR 2017/746/EU, IVDD 98/79/EC, Annex II, List A). However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[1,2]

### Handling

Carefully open one bottle, avoiding the loss of lyophilizate, and pipette in exactly 1.0 mL of distilled/deionized water. Carefully close the bottle and dissolve the contents completely by occasional gentle swirling within 30 minutes. Avoid the formation of foam.

The enclosed barcoded labels are intended exclusively for **cobas c** systems to identify the calibrator. Attach the barcoded labels to the tubes carrying the sample cups containing the calibrator material.

### Storage and stability

Store at 2-8 °C.

Criterion for the stability data stated by Roche:

Recovery within ± 10 % of initial value.

Stability of the lyophilized calibrator at 2-8 °C:

Up to the stated expiration date.

Stability of the components in the reconstituted calibrator:

| | |
|---|---|
| at 15-25 °C | 8 hours |
| at 2-8 °C | 5 days |
| at (-15)-(-25) °C | 4 weeks (when frozen once) |

Store calibrator tightly capped when not in use.

### Materials provided

- See "Reagents – working solutions" section
- Barcoded labels

### Materials required (but not provided)

- Roche system reagents and clinical chemistry analyzers
- General laboratory equipment

### Assay

Use C.f.a.s. Lipids as specified in the relevant Method Sheet for the system reagents.

### References

1   Occupational Safety and Health Standards: Bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.
2   Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

### Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

| CONTENT | Contents of kit |
|---|---|
| CALIBRATOR | Calibrator |

12172623500V9.0

# C.f.a.s. Lipids

**cobas®**

→ Volume for reconstitution

GTIN Global Trade Item Number

CE 0123

⬡ Roche

03555941500V7.0

# C.f.a.s. PAC

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| REF 03555941190    [i] 03555941500    → 3 x 1 mL Calibrator

## English

### System information

For use on **cobas c** and COBAS INTEGRA analyzer systems, refer to the corresponding method sheet of the assay for the identification on the systems.

### Intended use

C.f.a.s. (Calibrator for automated systems) PAC (Prealbumin-ASLO-Ceruloplasmin) is for use in the calibration of quantitative Roche methods on Roche clinical chemistry analyzers as specified in the value sheets.

### Summary

C.f.a.s. PAC is a lyophilized calibrator based on human serum.

The concentrations of the calibrator components have been adjusted to ensure optimal calibration of the appropriate Roche methods on clinical chemistry analyzers.

Some methods specified in the relevant value sheet may not be available in all countries.

### Reagents – working solutions

*Reactive components in the lyophilizate:*

Human serum with chemical additives and material of biological origin as specified. The origin of the biological additives is as follows:

| Analyte | Origin |
|---------|--------|
| ASLO | sheep |

*Non-reactive components:*

Preservative and stabilizer

The concentrations of the calibrator components are lot-specific. The exact calibrator values are given in the electronically available or enclosed value sheets.

The values are also encoded in the enclosed calibrator barcode sheets for COBAS INTEGRA analyzers.

For the **cobas c** analyzers (except for the **cobas c** 111 analyzer) the values are encoded in electronic files sent via the **cobas** link to the analyzers.

### Calibrator values

The calibrator values were determined using the method stated in the electronically available or enclosed value sheets. Determinations were performed under strictly standardized conditions on Roche analyzers using Roche system reagents and the Roche master calibrator.

The calibrator values were obtained via single determinations performed in different laboratories, in several separate runs. The calibrator value specified is the mean of all values obtained.

Traceability information is given in the relevant Method Sheets for the system reagents.

### Precautions and warnings

For in vitro diagnostic use for laboratory professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

CAUTION. WARNING. The bottles contain sodium azide (< 1 %). Avoid contact with skin and mucous membranes. Flush affected areas with copious amounts of water. Get immediate medical attention for eyes, or if ingested. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up. Clean exposed metal surfaces with 10 % sodium hydroxide.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

| H412 | Harmful to aquatic life with long lasting effects. |

**Prevention:**

| P273 | Avoid release to the environment. |

**Disposal:**

| P501 | Dispose of contents/container to an approved waste disposal plant. |

Product safety labeling primarily follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

All human material should be considered potentially infectious. All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods use assays that have been approved or cleared by the FDA or that are in compliance with the legal rules of the European Union (IVDR 2017/746/EU, IVDD 98/79/EC, Annex II, List A). However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[1,2]

### Handling

Carefully open one bottle, avoiding the loss of lyophilizate, and pipette in exactly 1.0 mL of distilled/deionized water. Carefully close the bottle and dissolve the contents completely by occasional gentle swirling within 30 minutes. Avoid the formation of foam.

The enclosed barcoded labels are intended exclusively for **cobas c** systems to identify the calibrator. Attach the barcoded labels to the tubes carrying the sample cups containing the calibrator material.

### Storage and stability

Store at 2-8 °C.

Criterion for the stability data stated by Roche:

Recovery within ± 10 % of initial value.

Stability of the lyophilized calibrator at 2-8 °C:

Up to the stated expiration date.

Stability of the components in the reconstituted calibrator:

| at 15-25 °C | 8 hours |
| at 2-8 °C | 2 days |
| at (-15)-(-25) °C | 2 weeks (when frozen once) |

Store calibrator tightly capped when not in use.

### Materials provided

- See "Reagents – working solutions" section
- Barcoded labels

### Materials required (but not provided)

- Roche system reagents and clinical chemistry analyzers
- General laboratory equipment

### Assay

Use C.f.a.s. PAC as specified in the relevant Method Sheet for the system reagents.

### References

1   Occupational Safety and Health Standards: Bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.

2   Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

The Summary of Safety & Performance Report can be found here: https://ec.europa.eu/tools/eudamed

# C.f.a.s. PAC

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## Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

| CONTENT | Contents of kit |
| CALIBRATOR | Calibrator |
| → | Volume for reconstitution |
| GTIN | Global Trade Item Number |

| Rx only | For USA: Caution: Federal law restricts this device to sale by or on the order of a physician. |

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All other product names and trademarks are the property of their respective owners.

Additions, deletions or changes are indicated by a change bar in the margin.

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CE 0123

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⬡ Roche

# C.f.a.s. Proteins

**cobas**®

| | | |
|---|---|---|
| REF 11355279216 | 11355279500 | 5 x 1 mL Calibrator |

## English

### System information

For use on **cobas c** and COBAS INTEGRA analyzer systems, refer to the corresponding method sheet of the assay for the identification on the systems.

### Intended use

C.f.a.s. (Calibrator for automated systems) Proteins is for use in the calibration of quantitative Roche methods on Roche clinical chemistry analyzers as specified in the value sheets.

### Summary

C.f.a.s. Proteins is a liquid ready-for-use calibrator based on human serum.

The concentrations of the calibrator components have been adjusted to ensure optimal calibration of the appropriate Roche methods on clinical chemistry analyzers.

Some methods specified in the relevant value sheet may not be available in all countries.

### Reagents – working solutions

*Reactive components:*

Human serum with chemical additives and material of biological origin as specified.

The origin of the biological additives is as follows:

| Analyte | Origin |
|---|---|
| Ferritin | human |
| CRP | human |

*Non-reactive components:*

Preservatives and stabilizer

The concentrations of the calibrator components are lot-specific. The exact calibrator values are given in the electronically available or enclosed value sheets.

The values are also encoded in the enclosed calibrator barcode sheets for COBAS INTEGRA analyzers.

For the **cobas c** analyzers (except for the **cobas c** 111 analyzer) the values are encoded in electronic files sent via the **cobas** link to the analyzers.

### Calibrator values

The calibrator values were determined using the method stated in the electronically available or enclosed value sheets. Determinations were performed under strictly standardized conditions on Roche analyzers using Roche system reagents and the Roche master calibrator.

The calibrator values were obtained via single determinations performed in different laboratories, in several separate runs. The calibrator value specified is the mean of all values obtained.

Traceability information is given in the relevant Method Sheets for the system reagents.

### Precautions and warnings

For in vitro diagnostic use for laboratory professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

⚠️

Warning

| | |
|---|---|
| H317 | May cause an allergic skin reaction. |

**Prevention:**

| | |
|---|---|
| P261 | Avoid breathing mist or vapours. |
| P272 | Contaminated work clothing should not be allowed out of the workplace. |
| P280 | Wear protective gloves. |

**Response:**

| | |
|---|---|
| P333 + P313 | If skin irritation or rash occurs: Get medical advice/attention. |
| P362 + P364 | Take off contaminated clothing and wash it before reuse. |

**Disposal:**

| | |
|---|---|
| P501 | Dispose of contents/container to an approved waste disposal plant. |

**Hazardous components:**

- 2-methyl-2H-isothiazol-3-one hydrochloride

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

All human material should be considered potentially infectious. All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods use assays that have been approved or cleared by the FDA or that are in compliance with the legal rules of the European Union (IVDR 2017/746/EU, IVDD 98/79/EC, Annex II, List A). However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[1,2]

### Handling

The product is ready-for-use. Mix carefully before use. Avoid the formation of foam.

The enclosed barcoded labels are intended exclusively for **cobas c** systems to identify the calibrator. Attach the barcoded labels to the tubes carrying the sample cups containing the calibrator material.

### Storage and stability

Store at 2-8 °C.

Criterion for stability data stated by Roche:
Recovery within ± 10 % of initial value.

Stability:

| | |
|---|---|
| Unopened: | Up to the stated expiration date at 2-8 °C. |
| After opening: | 4 weeks at 2-8 °C, provided that dispensing of the calibrator occurs without microbial contamination, e.g. by pouring out. |

Store calibrator tightly capped when not in use.

### Materials provided

- See "Reagents – working solutions" section
- Barcoded labels

### Materials required (but not provided)

- Roche system reagents and clinical chemistry analyzers
- General laboratory equipment

### Assay

Use C.f.a.s. Proteins as specified in the relevant Method Sheet for the system reagents.

# C.f.a.s. Proteins

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## References

1   Occupational Safety and Health Standards: Bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.

2   Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

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## Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

| CONTENT | Contents of kit |
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| Rx only | For USA: Caution: Federal law restricts this device to sale by or on the order of a physician. |

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