COD 11811 1 x 200 mL	COD 11812 1 x 500 mL
STORE AT 2-30ºC	
Reagents for measurement of calcium concentration Only for *in vitro* use in the clinical laboratory	

CALCIUM-CRESOLPHTHALEIN

BioSystems

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CALCIUM-CRESOLPHTHALEIN
O-CRESOLPHTHALEIN COMPLEXONE

PRINCIPLE OF THE METHOD

Calcium in the sample reacts with o-cresolphthalein complexone (o-CPC) forming a coloured complex that can be measured by spectrophotometry[1].

CONTENTS

	COD 11811	COD 11812
A. Reagent	1 x 160 mL	1 x 400 mL
B. Reagent	1 x 40 mL	2 x 50 mL
S. Standard	1 x 5 mL	1 x 5 mL

COMPOSITION

A. Reagent. Ethanolamine 900 mmol/L.

B. Reagent. o-Cresolphthalein Complexone 0.3 mmol/L, 8-hydroxyquinoline 28 mmol/L, hydrochloric acid 100 mmol/L.

DANGER: H314: Causes severe skin burns and eye damage. P280: Wear protective gloves/protective clothing/eye protection/face protection. P303+P361+P353: IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.

S. Calcium/Magnesium Standard. Calcium 10 mg/dL (2.5 mmol/L), magnesium 2 mg/dL. Aqueous primary standard.

For further warnings and precautions, see the product safety data sheet (SDS).

STORAGE

Store at 2-30ºC.

Reagents and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagents: Presence of particulate material, turbidity, absorbance of the blank over 0.500 at 560 nm.

– Standard: Presence of particulate material, turbidity.

WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents. Safety data sheet available for professional user on request. Disposal of all waste material should be in accordance with local guidelines.

REAGENT PREPARATION

Standard is provided ready to use.

Working Reagent (Note 1):

- Cod. 11811: Pour the contents of the Reagent B into the Reagent A bottle. Mix gently. Other volumes can be prepared in the proportion: 4 mL Reagent A + 1 mL Reagent B. Stable for 2 days at 2-8ºC.
- Cod. 11812: Transfer 100 mL of Reagent B into a Reagent A bottle. Mix gently. Other volumes can be prepared in the proportion: 4 mL Reagent A + 1 mL Reagent B. Stable for 2 days at 2-8ºC.

ADDITIONAL EQUIPMENT

– Analyzer, spectrophotometer or photometer able to read at 560 ± 20 nm.

SAMPLES

Serum, heparinized plasma or urine collected by standard procedures (Note 1).

Calcium in serum or plasma is stable for 10 days at 2-8ºC. Anticoagulants other than heparin should not be used.

Collect a 24-hour urine specimen in a bottle containing 10 mL of 50 % (v/v) nitric acid. Stable for 10 days at 2-8ºC. Centrifuge or filter and dilute ½ with distilled water before testing.

PROCEDURE

1. Pipette into labelled test tubes: (Notes 1,2)

	Blank	Standard	Sample
Calcium Standard (S)	—	13 µL	—
Sample	—	—	13 µL
Working Reagent	1.0 mL	1.0 mL	1.0 mL

2. Mix thoroughly and let stand the tubes for 4 minutes at room temperature.
3. Read the absorbance (A) of the Standard and the Sample at 560 nm against the Blank. The colour is stable for at least 1 hour.

CALCULATIONS

The calcium concentration in the sample is calculated using the following general formula:

$$\frac{A_{Sample}}{A_{Standard}} \times C_{Standard} \times \text{Sample dilution factor} = C_{Sample}$$

If the Calcium Standard provided has been used to calibrate (Note 3):

	Serum and plasma	Urine
$\frac{A_{Sample}}{A_{Standard}}$	x 10 = mg/dL calcium x 2.5 = mmol/L calcium	x 20 = mg/dL calcium x 5 = mmol/L calcium

REFERENCE VALUES

Serum and plasma[2]: 8.6 - 10.0 mg/dL = 2.15 - 2.50 mmol/L

Urine[2]: 100 - 300 mg/24-h = 2.5 - 7.5 mmol/24-h

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, cod. 18009 and cod. 18042), level II (cod. 18007, cod. 18010 and cod. 18043) and the Biochemistry Control Urine (cod. 18054 and cod. 18066) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

– Detection limit: 0.26 mg/dL calcium = 0.06 mmol/L calcium.

– Linearity limit: 20 mg/dL calcium = 5 mmol/L calcium. For higher values dilute sample 1/2 with distilled water and repeat measurement.

– Repeatibility (within run):

Mean calcium concentration	CV	n
9.58 mg/dL = 2.40 mmol/L	1.7 %	20
13.6 mg/dL = 3.40 mmol/L	1.4 %	20

– Reproducibility (run to run):

Mean calcium concentration	CV	n
9.58 mg/dL = 2.40 mmol/L	2.2 %	25
13.6 mg/dL = 3.40 mmol/L	1.6 %	25

– Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 3). Details of the comparison experiments are available on request.

– Interferences: Bilirubin (< 20 mg/dL), hemolysis (hemoglobin < 10 g/L) and lipemia (triglycerydes < 30 g/L) do not interfere. Other drugs and substances may interfere[3].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Calcium is the most prevalent cation found in the body, distributed in bone (99%), soft tissues and extracellular fluid. Its concentration in plasma is regulated by parathyroid hormone, vitamin D and calcitonin.

Calcium ion is important in the transmission of nerve impulses, in the maintenance of normal muscle contractility, as a cofactor in certain enzyme reactions, and in the coagulation of the blood.

Hypercalcemia can be due to vitamin D intoxication, enhanced renal retention, osteoporosis, sarcosidosis, thyrotoxicosis, hyperparathyroidsm, multiple mieloma, idiopathic hypercalcemia of infancy, and carcinoma metastasic to bone[2,4].

Elevated calcium concentration in urine is found in nephrolithiasis and metabolic acidosis[2,4].

Hypocalcemia may be caused by primary and secondary hypoparathyroidism, pseudohypoparathyroidism, vitamin D deficiency, malnutrition and intestinal malabsorption[2,4].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. Contamination of glassware with calcium will affect the test. Use acid-washed glassware or plastic tubes.
2. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.
3. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

BIBLIOGRAPHY

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