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Hormone

AFIAS β-HCG Plus

INTENDED USE

AFIAS β-HCG Plus is a fluorescence immunoassay (FIA) for the quantitative determination of human Total β-hCG (chorionic gonadotrophin) in human whole blood/serum/plasma. It is useful as an aid in management and monitoring of fertility.

For *in vitro* diagnostic use only.

INTRODUCTION

Human chorionic gonadotropin (hCG) is a glycoprotein hormone secreted by the developing placenta shortly after implantation. hCG can be detected in the urine and serum of pregnant women as early as 6 to 15 days after conception. The concentration of hCG increases to 50 mIU/mL one week post implantation and reaches to about 100 mIU/mL at the time of the first missed menstrual period and the peak at 100,000-200,000 mIU/mL at the first trimester.

PRINCIPLE

The test uses a sandwich immunodetection method.

The detector anti-βhCG and capture anti-βhCG in buffer bind to β-hCG in the sample forming antigen-antibody complexes and migrates onto nitrocellulose matrix to be captured by the other immobilized streptavidin on a test strip.

More β-hCG in the sample will form more antigen-antibody complexes which lead to stronger fluorescence signal by detector anti-βhCG, which is processed by the instrument for AFIAS tests to show total β-hCG concentration in the sample.

COMPONENTS

AFIAS β-HCG Plus consists of 'cartridges'.

- Each sealed aluminum pouch contains two cartridges.
- Each cartridge packaged in an aluminum pouch has three components including a cartridge part, a detector part and a diluent part.
- The cartridge contains the membrane called a test strip which has streptavidin at two test lines, mouse IgG-hCG peptide at the antigen line, and chicken IgY at the control line.
- The detector part has two granules containing anti-hCG-fluorescence conjugate, anti-hCG-biotin conjugate, anti-chicken IgY-fluorescence conjugate, and sodium azide as a preservative in Tris buffer and phosphate buffered saline (PBS).
- The diluent part contains sodium azide as a preservative and tween 20 as a detergent in phosphate buffered saline (PBS).

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow instructions and procedures described in this 'Instructions for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge and ID chip must match each other.
- Do not interchange test components between different lots or use test components after the expiration date, either of which might yield incorrect test result(s).
- Do not reuse cartridges. A cartridge should be used for testing one sample only.
- The cartridge should remain sealed in its original pouch until just before use. Do not use a cartridge, if the pouch is damaged or has already been opened.
- Frozen samples should be thawed only once. For shipping, samples must be packed in accordance with local regulations. Sample with severe hemolysis and/or hyperlipidemia must not be used.
- If test components and/or sample are stored in refrigerator, then allow cartridge and sample to be at room temperature for approximately 30 minutes before use.
- The instrument for AFIAS tests may generate slight vibration during use.
- Used cartridges, C-tips and pipette tips pipette tips should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- The cartridge contains sodium azide (NaN_3), and it may cause certain health issues like convulsions, low blood pressure, low heart rate, loss of consciousness, lung injury and respiratory failure. Avoid contact with skin, eyes, and clothing. In case of contact, rinse immediately with running water.
- No Biotin interference was observed in **AFIAS β-HCG Plus** when biotin concentration in the sample was below 500 ng/mL. If a patient has been taking biotin, it is recommended to test again 24 hours after discontinuation of biotin intake.
- **AFIAS β-HCG Plus** will provide accurate and reliable results subject to the below conditions.
  - **AFIAS β-HCG Plus** should be used only in conjunction with the instrument for AFIAS tests.
  - Have to use recommended anticoagulant.

Recommended anticoagulant
K_2 EDTA, K_3 EDTA, Sodium heparin

- **C-tip should be used when the following conditions are met.**
  - C-tip provided with the kit is recommended to obtain correct test result.
  - Whole blood should be immediately tested after collection.
  - Do not perform a test with C-tip on General Mode. It might cause an erroneous result.
  - Excess whole blood around the C-tip should be wiped off.
  - In order to avoid cross-contamination, please do not re-use C-tip for multiple samples.
  - AFIAS cartridges should be inserted and positioned in the cartridge holder prior to the blood sample collection.
  - While collecting blood, be careful not to create air bubbles in the C-tip.

LIMITATIONS OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result(s) due to the non-responsiveness of the antigens to the antibodies which is the most common if the epitope is masked by some unknown components, so therefore not being able to be detected or captured by the antibodies. The instability or degradation of the antigens with time and/or temperature may also cause the false negative result as it makes antigens unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician in conjunction with clinical symptoms and other relevant test results.

STORAGE AND STABILITY

Storage condition			
Component	Storage Temperature	Shelf life	Note
Cartridge	2 - 30°C	20 months	Unopened
		1 month	Resealed

- Return an unused cartridge to the spare cartridge zipper bag containing the desiccant pack. Reseal along entire edge of zip-seal.

MATERIALS SUPPLIED

REF SMFP-52

Components of **AFIAS β-HCG Plus**

- Cartridge box:
  - Cartridge 24
  - Pipette tip (zipper bag) 24
  - C-tip (30 µL) 24
  - Spare cartridge zipper bag 1
  - ID chip 1
  - Instructions for use 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately with **AFIAS β-HCG Plus**.

Please contact our sales division for more information.

- **Instrument for AFIAS tests**
  - **AFIAS-1** REF FPRR019
  - **AFIAS-3** REF FPRR040
  - **AFIAS-6** REF FPRR020
  - **AFIAS-10** REF FPRR038
- **Boditech hCG Plus Control** REF CFPO-233
- **Boditech hCG Plus Calibrator** REF CFPO-259

SAMPLE COLLECTION AND PROCESSING

The sample type for **AFIAS β-HCG Plus** is human whole blood/serum/plasma.

- It is recommended to test the sample within 24 hours after collection when collected sample is stored at room temperature.
- The samples (serum, plasma) should be separated from the clot by centrifugation within 3 hours after the collection of whole blood.
- The samples (whole blood, serum, plasma) may be stored for a week at 2-8°C prior to being tested. If testing will be delayed more than a week, samples should be frozen at -20°C.
- The samples (serum, plasma) stored frozen at -20°C for 3 months showed no performance difference.
- However, the whole blood sample should not be kept in a freezer in any case.
- As a repeated freeze-thaw cycle may affect the test result, do not refreeze previously frozen samples.
- Collection of whole blood sample using C-tip.
  ① Hold the C-tip horizontally and touch the surface of the blood with the tip of the C-tip.
  ② Capillary action will automatically draw the blood sample to C-tip and stop.
  ③ Wipe off any excess blood around the tip.
  ④ Double-check if whole blood is filled accurately in the C-tip and the instrument for AFIAS tests is ready for a test on the 'C-tip mode'.

TEST SETUP

- Check the components of the **AFIAS β-HCG Plus** as described below: Cartridges, pipette tips, C-tips, an ID chip, a spare cartridge zipper bag and an instructions for use.
- Ensure that the lot number of the cartridge matches that of the ID chip.
- If the sealed cartridge has been stored in a refrigerator, place them on a clean and flat surface at room temperature for at least 30 minutes before testing.
- Turn on the instrument for AFIAS tests.
- Empty the tip box.
- Insert the ID chip into the 'ID chip port'.

※ **Please refer to the instrument for AFIAS tests operation manual for complete information and operating instructions.**

TEST PROCEDURE

▶ **AFIAS-1, AFIAS-3, AFIAS-6**

General mode

1) Insert a cartridge into the cartridge holder.
2) Insert a tip into the tip hole of the cartridge.
3) Select the 'General mode' in the instrument for AFIAS tests.
4) Take 150 µL of sample (whole blood/serum/plasma/ control) using a pipette and dispense it into the sample well of the cartridge.
5) Tap the 'Start' button on the screen.
6) The test result will be displayed on the screen after 12 minutes.

C-tip mode

1) Insert a cartridge into the cartridge holder.
2) Take 30 µL of whole blood using a C-tip.
3) Insert the whole blood-filled C-tip into the tip hole of the cartridge.
4) Select the 'C-tip mode' in the instrument for AFIAS tests.
5) Tap the 'Start' button on the screen.
6) The test result will be displayed on the screen after 12 minutes.

▶ **AFIAS-10**

Normal mode

1) Insert a cartridge into the cartridge holder.
2) Insert a tip into the tip hole of the cartridge.
3) Tap the 'Load' button of the bay that holds the cartridge with the tip to read the barcode of the cartridge and please confirm the item name written on the tube rack.
4) Insert the sample tube into the tube rack.
5) Insert the tube rack into the loading part of the sampling station.
6) Tap the 'Start' button on the screen.
7) The test result will be displayed on the screen after 12

minutes.

Emergency mode – General tip

1) The test procedure is same with the 'Normal mode 1) – 3)'.
2) Convert the 'Emergency mode' in AFIAS-10.
3) Select the tip type (general tip) on the screen.
4) Select the sample type (whole blood/serum/plasma) on the screen.
5) Take 150 µL of the sample using a pipette and dispense it into the sample well of the cartridge.
6) Tap the 'Start' button on the screen.
7) The test result will be displayed on the screen after 12 minutes.

Emergency mode – C-tip

1) Insert a cartridge into the cartridge holder.
2) Take 30 µL of whole blood using a C-tip.
3) Insert the C-tip with sample into the tip hole of the cartridge.
4) Tap the 'Load' button of the bay that holds the cartridge with a tip to read the barcode of the cartridge and please confirm the item name written on the cartridge.
5) Convert the 'Emergency mode' in AFIAS-10.
6) Select the tip type (C-tip) on the screen.
7) Tap the 'Start' button on the screen.
8) The test result will be displayed on the screen after 12 minutes.

INTERPRETATION OF TEST RESULT

- The instrument for AFIAS tests calculates the test result automatically and displays total β-hCG concentration of the test sample in terms of mIU/mL.
- Reference range

Pregnant women (week since LMP*)	Total β-hCG level [mIU/mL]
3 weeks	5-50
4 weeks	5-426
5 weeks	18-7,340
6 weeks	1,080-56,500
7-8 weeks	7,650-229,000
9-12 weeks	25,700-288,000
13-16 weeks	13,000-254,000
17-24 weeks	4,060-165,400
25-40 weeks	3,640-117,000

- Working range: 2-1,500 mIU/mL

QUALITY CONTROL

- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are not provided on demand with **AFIAS β-HCG Plus**. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance.
  (Please refer to the instructions for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical sensitivity**
  - Limit of Blank (LoB) 0.10 mIU/mL
  - Limit of Detection (LoD) 0.50 mIU/mL
  - Limit of Quantitation (LoQ) 1.80 mIU/mL

- **Analytical specificity**
  - Cross-reactivity
    Biomolecules such as below the ones in the table were added to the test sample(s) at concentrations much higher than their normal physiological levels in the blood. **AFIAS β-HCG Plus** test results did not show any significant cross-reactivity with these biomolecules.

Cross-reactants	Concentration
hTSH	500 µIU/mL
hFSH	1,000 mIU/mL
hLH	1,000 mIU/mL

  - Interference
    Interferents listed in the following table were added to the test sample at the concentration mentioned below. **AFIAS β-HCG Plus** test results did not show any significant interference with these materials.

Interferents	Concentration
D-Glucose	60 mM/L
L-Ascorbic acid	0.2 mM/L
Bilirubin	0.4 mM/L
Hemoglobin	2 g/L
Cholesterol	13 mM/L
Triglyceride	40 mM/L

  - Subunit reactivity
    AFIAS β-HCG Plus does not have the capability to detect hCG α subunit and β core fragment.

- **Precision**
  - **Single-site study**
    Repeatability (within-run precision)
    within-laboratory precision (Total precision)
    Lot to lot precision
    3 Lots of **AFIAS β-HCG Plus** were tested for 20 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.

Single-site study						
Total β-hCG [mIU/mL]	Repeatability		within-laboratory precision		Lot to lot precision	
	AVG [mIU/mL]	CV (%)	AVG [mIU/mL]	CV (%)	AVG [mIU/mL]	CV (%)
20	20.29	7.5	20.30	7.8	20.29	8.2
100	100.80	7.3	100.39	7.9	100.21	7.8
900	889.52	8.4	892.76	7.8	902.90	7.7

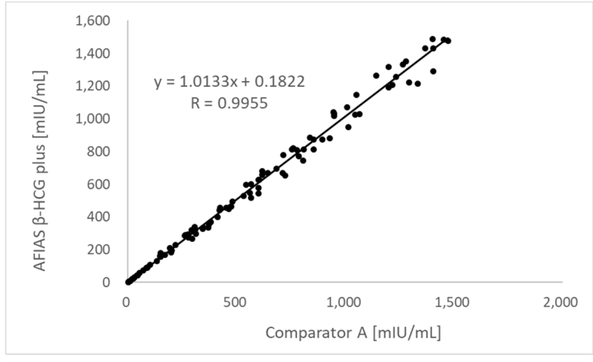
  - **Multi-site study**
    Reproducibility
    1 Lot of **AFIAS β-HCG Plus** was tested for 5 days in 3 different sites (1 person per 1 site, 1 instrument per 1 site). Each standard material was tested 1 time per and 5 replicates per day.

Multi-site study		
Total β-hCG [mIU/mL]	Reproducibility	
	AVG [mIU/mL]	CV (%)
20	19.96	8.0
100	100.69	7.2
900	888.67	8.3

- **Accuracy**
  The accuracy was confirmed by testing with 3 different lots of **AFIAS β-HCG Plus.** The tests were repeated 10 times at each concentration of the control standard.

Total β-hCG [mIU/mL]	Lot 1	Lot 2	Lot 3	AVG	Recovery (%)
5	4.95	5.01	5.11	5.02	100
20	19.97	19.71	20.80	20.16	101
100	99.46	103.11	98.75	100.44	100
400	392.92	421.81	403.02	405.92	101
1000	963.50	1031.50	979.20	991.40	99
1400	1386.46	1415.68	1438.89	1413.68	101

- **Comparability**
  Total β-hCG concentration of 100 clinical samples was quantified independently with **AFIAS β-HCG Plus (AFIAS-6)** and **Comparator A** as per prescribed test procedures. Test results were compared, and their comparability was investigated with linear regression and correlation coefficient (R). The regression equation and correlation coefficient are as follows.



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Note: Please refer to the table below to identify various symbols.

Σ	Sufficient for <n> tests
	Read instruction for use
	Use by Date
LOT	Batch code
REF	Catalog number
	Caution
	Manufacturer
EC REP	Authorized representative of the European Community
IVD	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
CE	This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices

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