Hormone

AFIAS Progesterone

INTENDED USE

AFIAS Progesterone is a fluorescence immunoassay (FIA) for the quantitative determination of Progesterone in human serum/plasma. It is useful as an aid in management and monitoring of the cause of infertility, track ovulation, diagnose an ectopic or failing pregnancy, monitor the health of a pregnancy.

For *in vitro* diagnostic use only.

INTRODUCTION

Progesterone also known as P4 (pregn-4-ene-3,20-dione) is a C-21 steroid hormone involved in the female menstrual cycle, pregnancy (supports gestation) and embryogenesis of humans and other species.[2] Progesterone belongs to a class of hormones called progestogens, and is the major naturally occurring human progestogen.

In mammals, progesterone, like all other steroid hormones, is synthesized from pregnenolone, which in turn is derived from cholesterol.

Progesterone is essential for the regulation of normal female reproductive functions. The major physiological actions of progesterone are: a) in the uterus and ovary: induction of ovulation, facilitation of implantation, and maintenance of early pregnancy; b) in the mammary gland: lobular-alveolar development in preparation for milk secretion[3,4]; c) in the brain: neurobehavioral expression associated with sexual responsiveness[5] and d) in the bone: prevention of bone loss[6]. During the follicular phase of the cycle, progesterone levels remain low[7-9]. Following the LH surge and ovulation, luteal cells in the ruptured follicle produce progesterone in response to LH. During this, the luteal phase, progesterone rises rapidly to a maximum of 10-20 ng/mL at day 5-7 following ovulation. During the luteal phase, progesterone transforms the estrogen-primed endometrium from a proliferative to a secretory state.[8] If pregnancy does not occur, progesterone levels decrease during the last four days of the cycle due to the regression of the corpus luteum.[7,8-13] If conception occurs, the levels of progesterone are maintained at mid-luteal levels by the corpus luteum until about week six. At that time the placenta becomes the main source of progesterone and levels rise from approximately 10-50 ng/mL in the first trimester to approximately 50-280 ng/mL in the third trimester.[7,14,15]

PRINCIPLE

The test uses a competitive immunodetection method.

The antigens in the sample binds to the fluorescence-labeled detector antibodies in buffer, forming the complexes as a sample mixture. They will migrate onto nitrocellulose matrix, which will interfere with the binding of the free fluorescence-labeled detector antibodies to the immobilized-BSA-progesterone conjugate on the test strip.

More antigens in the sample will result in less free detection antibodies to accumulate, which lead to less fluorescence signal by the free fluorescence-labeled detector antibodies. This signal is processed by the instrument for AFIAS tests to show Progesterone concentration in the sample.

COMPONENTS

AFIAS Progesterone 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 part contains the membrane called a test strip which has BSA-progesterone conjugate and anti-mouse IgG at the test lines, and streptavidin at the control line.
- The detector part has a granule containing anti-human progesterone fluorescence conjugate, biotin-BSA fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide as a preservative in phosphate buffered saline (PBS).
- The diluent part contains bovine serum albumin (BSA) as a stabilizer, tween20 as a detergent and sodium azide as a preservative 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 the test components between different lots or use the 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 sample 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 and 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 they may cause certain health issues like convulsions, low blood pressure and 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 Progesterone** when biotin concentration in the sample was below 500 ng/mL. If a patient has been taking biotin at dosage of more than 0.03 mg a day, it is recommended to test again 24 hours after discontinuation of biotin intake.
- **AFIAS Progesterone** will provide accurate and reliable results subject to the below conditions.
  - **AFIAS Progesterone** should be used only in conjunction with instrument for AFIAS tests.
  - Have to use recommended anticoagulant.

Recommended anticoagulant
K_2 EDTA, K_3 EDTA, Lithium heparin

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 antigen 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 antigen with time and/or temperature may also cause false negative result as it makes the antigen 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-94

Components of **AFIAS Progesterone**

- Cartridge box:
  - Cartridge 24
  - Pipette tip (zipper bag) 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 from **AFIAS Progesterone.**

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 Hormone Control** REF CFPO-95
- **Boditech Hormone Calibrator** REF CFPO-107
- **Boditech Progesterone Control** REF CFPO-238
- **Boditech Progesterone Calibrator** REF CFPO-264

SAMPLE COLLECTION AND PROCESSING

The sample type for **AFIAS Progesterone** is human serum/plasma.

- The samples (serum, plasma) should be separated from the clot by centrifugation within 3 hours after the collection of whole blood.
- The samples (serum, plasma) must be tested within 12 hours after collection if they are kept at room temperature.
- The samples (serum, plasma) may be stored for 3 days at 2-8 °C prior to being tested. If testing will be delayed more than 3 days, samples (serum, plasma) should be frozen at -20 °C.
- The samples (serum, plasma) stored frozen at -20 °C for 1 months showed no performance difference.
- When the sample is thawed, shake gently and mix to use.
- As a repeated freeze-thaw cycle may affect the test result, do not refreeze previously frozen samples.

TEST SETUP

- Check the components of the **AFIAS Progesterone** as described below: cartridges, pipette tips, an ID chip, a spare cartridge zipper bag and an instructions for use.
- 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 the 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 100 µL of the sample (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 15 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 cartridge.
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 15 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 (serum/plasma) on the screen.
5) Take 100 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 15 minutes.

INTERPRETATION OF TEST RESULT

- The instrument for AFIAS tests calculates the test result automatically and displays Progesterone concentration of the test sample in terms of nmol/L.
- Reference range

Type		Range [nmol/L]
Males		4.56-5.19
Females	Mid-follicular phase	4.72-9.25
	Mid-luteal phase	20.73-56.13
	Post-menopausal	< 4.45
	Pregnancy	33.64-126.16

- Working range: 4.45 - 127.2 nmol/L (1.4 - 40 ng/mL)
- Conversion factor: 1 ng/mL = 3.18 nmol/L

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 provided on demand with **AFIAS Progesterone**. 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) 1.27 nmol/L
  - Limit of Detection (LoD) 2.37 nmol/L
  - Limit of Quantitation (LoQ) 4.45 nmol/L
- **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 Progesterone** test results did not show any significant cross-reactivity with these biomolecules.

Cross reactivity materials	Conc.
17-α-OH-progesterone	2 µg/mL
17β-estradiol(estradiol)	2 µg/mL
5α-pregnane-3,20-dione	0.2 µg/mL
Hydrocortisone	2 µg/mL
Danazol	20 µg/mL
Estriol	2 µg/mL
Testosterone	2 µg/mL
Dexamethasone	2 µg/mL
Estrone	2 µg/mL
Transferrin	2 µg/mL

- **Interference**

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

Interference materials	Conc.
D-glucose	600 mM
L-Ascorbic acid	2 mM
Bilirubin [unconjugate]	4 mM
Hemoglobin (human)	20 g/L
Cholesterol	130 mM
triglyceride	100 mg/ml

- **Precision**
  - Single-site study

Repeatability (within-run precision)
within-laboratory precision (Total precision)
Lot to lot precision

3 Lots of **AFIAS Progesterone** were tested for 20 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.

  - Multi-site study

Reproducibility

1 Lot of **AFIAS Progesterone** 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.

Conc. [nmol/L]	Single-site study					
	Repeatability			Within-laboratory precision		
	Mean	SD	CV (%)	Mean	SD	CV (%)
12.72	12.63	1.45	11.46	12.79	1.33	10.38
31.8	31.07	3.59	11.55	31.11	3.55	11.41
63.6	65.13	6.76	10.38	64.01	6.78	10.59
Conc. [nmol/]	Single-site study			Multi-site study		
	Lot to lot precision			Reproducibility		
	Mean	SD	CV (%)	Mean	SD	CV (%)
12.72	12.71	1.45	11.41	12.82	1.50	11.69
31.8	31.62	3.65	11.56	31.92	3.51	10.98
63.6	63.54	7.30	11.48	65.51	7.58	11.56

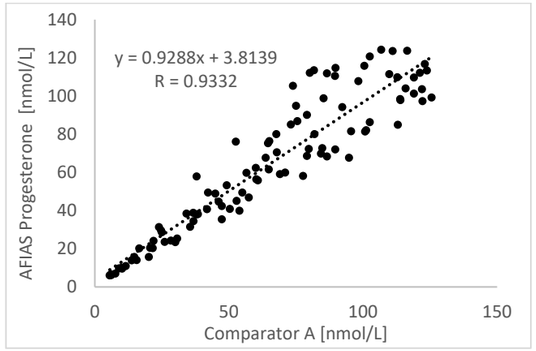
- **Accuracy**

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

Expected value [nmol/L]	Lot 1	Lot 2	Lot 3	AVG	Recovery
4.6	4.60	4.57	4.90	4.69	102 %
7.6	7.48	7.79	7.56	7.61	100 %
11.4	11.28	11.53	11.70	11.50	100 %
22.9	22.43	22.14	22.15	22.24	97 %
38.2	37.94	36.94	37.72	37.53	98 %
47.7	48.69	49.62	48.91	49.08	103 %
68.7	66.77	69.26	69.58	68.54	100 %
76.3	75.54	76.40	78.74	76.89	101 %
95.4	92.24	91.96	93.70	92.63	97 %

- **Comparability**

Progesterone concentration of 100 clinical samples were quantified independently with **AFIAS Progesterone (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

For technical assistance, please contact:
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