PROGESTERONE ENZYME IMMUNOASSAY TEST KIT
Catalog Number: 6107620

CE

Enzyme Immunoassay for the Quantitative Determination of Progesterone Concentration in Human Serum or Plasma

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2 to 8°C.

INTENDED USE

For the quantitative determination of Progesterone concentration in human serum or plasma

INTRODUCTION

Progesterone is a C21 steroid which is synthesized from both tissue and circulating cholesterol. Cholesterol is transformed to pregnenolone which is then converted via a combined dehydrogenase and isomerase to progesterone. The principle production sites are the adrenals and ovaries and the placenta during pregnancy. The majority of this steroid is metabolized in the liver to pregnanediol and conjugated as a glucuronide prior to excretion by the kidneys.

Progesterone exhibits a wide variety of end organ effects. The primary role of progesterone is exhibited by the reproductive organs. In males, progesterone is a necessary intermediate for the production of corticosteroids and androgens. In females, progesterone remains relatively constant throughout the follicular phase of the menstrual cycle. The concentration then increases rapidly following ovulation and remains elevated for 4-6 days and decreases to the initial level 24 hours before the onset of menstruation. In pregnancy, placental progesterone production rises steadily to levels of 10 to 20 times those of the luteal phase peak.

Progesterone measurements are thus performed to determine ovulation as well as to characterize luteal phase defects. Monitoring of progesterone therapy and early stage pregnancy evaluations comprise the remaining uses of progesterone assays.

The Progesterone EIA kits are designed for the measurement of total progesterone in human serum or plasma.

PRINCIPLE OF THE TEST

Progesterone (antigen) in the sample competes with horseradish peroxidase-progesterone (enzyme-labeled antigen) for binding onto the limited number of anti-progesterone (antibody) sites on the microplates (solid phase).

After incubation the bound/free separation is performed by a simple solid-phase washing.

The substrate solution B (H2O2) and the substrate solution A (TMB) are added. After an appropriate time has elapsed for maximum color development, the enzyme reaction is stopped and the absorbances are determinated.

Progesterone concentration in the sample is calculated based on a series of standards.

The color intensity is inversely proportional to the progesterone concentration of the sample.

REAGENTS

Materials provided with the kit:

- Microwell plate. 12x8 well strips. Individually separable wells. Coated with Anti-Progesterone IgG adsorbed on microplate., packaged in an aluminum bag with a drying agent.
- Calibrators. 5 Vials x 1 mL. (C0,C1,C2,C3,C4):

  Concentration of Progesterone:

	C_0	C_1	C_2	C_3	C_4
ng/mL	0	0.2	1.0	8.0	40.0

- Enzyme-antigen Conjugate. Progesterone-horseradish peroxidase (HRP) conjugate. 6 mL.
- TMB Substrate solution. H_2O_2-TMB 0.25 g/L (avoid any skin contact). 12 mL
- Stop Solution. Sulphuric acid 0.15 mol/L (corrosive: avoid any skin contact). 12 mL

Materials required but not provided:

- Multichannel pipettes and micropipettes (Precision ≥1.5%) and disposable tips.
- Microplate reader with a 450 nm filter. Reference filter of 620 or 655 nm is advisable.
- Manual or automated wash system.
- Absorbent paper of blotting the microplate wells.
- Distilled or deionised water.
- Timer.

STORAGE OF TEST KITS

The components will remain stable through the expiration date shown on the label if stored between 2-8°C in dark. Do not frezee. Do not use reagents beyond the kit expiration date.
The bag containing the microplate should be brought to room temperature before opening to avoid condensation in the wells.
Once opened the bag, microplate strips are stable for 1 month at 2-8°C in the plastic bag tightly sealed, with the silicagel.
Opened reagents are stable for 1 month at 2-8°C.

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REAGENT PREPARATION

CALIBRATORS

- Calibrators, before use mix for 2 min. with a vortex.
- Stable until the expiration date of the kit when stored at + 4ºC.

SPECIMEN COLLECTION AND PREPARATION

The determination of Progesterone can be performed in plasma as well as in serum .
Store reagent at -20°C if the determination is not performed on the same day of the sample collection.

Precaution

1. Do not use heavily hemolized samples.
2. Maximum precision is required for reconstitution and dispensation of the reagents.
3. This method allows the determination of Progesterone from 0.2 ng/mL to 40.0 ng/mL.
4. For higher values, for example in pregnancy, dilute the sample; consider the diluting factor when calculating the result.
5. The clinical significance of the Progesterone determination can be invalidated if the patient was treated with cortisone or natural or synthetic steroids.

ASSAY PROCEDURE

As it is necessary to perform the determination in duplicate, prepare two wells for each of the five points of the calibration curve (C_0-C_4), and for each sample, one for Blank.

1. Pipette:

	Calibrator	Sample	Blank
Sample	---	50 µL	---
Calibrators C_0 –C_4	50 µL	---	---
Conjugate	50 µL	50 µL	---

2. Incubate at 37°C for 1 hour.
3. Remove the contents from each well; wash the wells with 300 µL of distilled water.
4. Repeat the washing procedure draining the water completely.
5. Pipette

	Calibrator	Sample	Blank
Substrate Solution	100µL	100µL	100µL

6. Incubate at room temperature (20-25°C) for 15 minutes in the dark.
7. Pipette:

	Calibrator	Sample	Blank
Stop solution	100µL	100µL	100µL

8. Read the absorbance (E) at 450 nm against Blank.

CALCULATION OF RESULTS

Mean absorbance and relative percentage
Calculate the mean of the absorbances (Em) corresponding to the single points to the calibrator curve and of each sample. Express data as the percentage of the mean absorbance of B_0 (EmS_0) with the following formula:

$$(B/B_0)\% = \frac{Em}{(Em\ S_0)} \times 100$$

Calibrator curve
Plot the values of the standards expressed as (B/B0)% on the logit-log paper. Extrapolate the line passing through the points.

Calculation of results
Interpolate the values of the samples expressed as (B/B0)% on the standard curve to obtain the corresponding values of the concentrations expressed in ng/mL.

EXPECTED VALUES

Each laboratory must establish its own normal ranges based on patient population.

Men:			0.4 - 0.9 ng/mL
Women:	follicular phase		0.4 - 1.7 ng/mL
	midluteinic phase		4.9 - 18.8 ng/mL
	pregnancy	Weeks	(ng/mL)
		18-21	53-76
		22-25	60-86
		26-29	71-133
		30-33	86-142
		34-37	104-175
		38-41	117-187

PERFORMANCE CHARACTERISTICS

Specificity

The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

Progesterone	100 %
11 α-OH –progesterone	18 %
17 α-OH progesterone	16 %
20 α-OH progesterone	1 %
Estradiol	$< 1x10^{-2}$ %
Testosterone	$< 1x10^{-2}$ %
Cortisol	$< 1x10^{-3}$ %
Cholesterol	$< 1x10^{-3}$ %

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Precision

The inter and intra-run precision had a coefficient of variation of 4.8% and 2.9% respectively.

Sensitivity

The sensitivity of this method, calculated as two times the S.D. from B0, is 2 pg when the value of (B/B0)% is approx 90%.

Accuracy

The recovery of 0.2 - 1.0 - 8.0 - 40.0 ng/mL of Progesterone added to "plasma-free" sample gave an average value (±SE) of 106% ± 4.2% with reference to the original concentrations.

Correlation with RIA

Correlation with RIA performed on the same samples:

Y= -0.226 + 0.965x

r =0.996

n=25

This method allows the determination of Progesterone from 0.2 ng/ml to 40.0 ng/ml.

REFERENCES

1. Wisdom, G.B. Clin. Chem. 22 (8), 1243- 1255 (1976)
2. De Villa, G.O., et al J. Clin. Endocr. Metab.35, 458 (1972)
3. Hubl, W., Fehert, T., Ronde, W., Dormer, G., Taubert, H.H, Freymann, E. Endokrinologie, 1982, 79 (2), 165
4. Arakawa, H., Maeda, M., Tsuji, A., Chem. Pharm. Tokyo (1982) 30 8, 3036
5. D. Riad – Fanny, G.F. Read, R.F. Walker and K. Griffiths. Endocr. Rewiews, vol. 3 N° 4 (1982) 304 367.

QUALITY SYSTEM CERTIFIED
ISO 9001 ISO 13485

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