AccuDiag™ 17 α OH Progesterone ELISA Kit

REF 1292-17

IVD 96 Tests

Test	17 α OH Progesterone
Method	Enzyme Linked Immunosorbent Assay
Principle	Competitive Enzyme Immunoassay
Detection Range	0-19.2 ng /ml
Sample	25 µL serum / plasma
Total Time	~75 min.
Shelf Life	12 Months from the manufacturing date
Specificity	100 %
Sensitivity	0.05 ng/mL

INTENDED USE

Competitive immunoenzymatic colorimetric method for quantitative determination of 17α OH Progesterone concentration in serum and plasma. 17OH progesterone ELISA kit is intended for laboratory use only.

SUMMARY AND EXPLANATION

17-Hydroxyprogesterone (17-OH progesterone or 17OHP) is a C-21 steroid hormone produced in the adrenal gland and gonads, during the synthesis of glucocorticoids and sex steroids. It is derived from progesterone via 17-hydroxylase, a P450c17 enzyme, or from 17-hydroxypregnenolone via 3β-hydroxysteroid dehydrogenase/Δ^{5-4} isomerase. 17OH progesterone is a precursor of cortisol that accumulates in the case of adrenal 21-hydroxylase deficiency and decreased after replacement therapy with cortisol. High values are found in congenital adrenal hyperplasia.

17α -OHP has no defined physiologic role except as a precursor molecule.
Serum 17α -OHP levels are age-dependent, with peak levels observed during fetal life and the immediate postnatal period. During the first week of life, serum 17α -OHP levels fall ~50-fold as compared to cord blood values. A small transient increase occurs in male infants 30-60 days postnatally. Levels for both sexes remain at constant low levels during childhood, and then progressively increase during puberty reaching adult levels of ~100 ng/dL (~3.03 nmol/L). As with cortisol, serum 17α -OHP levels normally have an ACTH-dependent diurnal variation, with peak levels in the morning and a nadir at night. In addition, ovarian production of 17α -OHP increases during the luteal phase of the menstrual cycle.

17-hydroxyprogesterone is a natural progestin and in pregnancy increases in the third trimester primarily due to fetal adrenal production.
Normal levels are 3-90 ng/dl in children and in women, 15-70 ng/dl prior to ovulation, and 35-290 ng/dl during the luteal phase.

Measurements of levels of 17-hydroxyprogesterone are useful in the evaluation of patients with suspected congenital adrenal hyperplasia as the typical enzymes that are defective, namely 21-hydroxylase and 11β-hydroxilase, lead to a build-up of 17OHP. In contrast, the rare patient with 17α-hydroxylase deficiency will have very low or undetectable levels of 17OHP. Elevated serum 17 α -OHP levels at baseline and/or after ACTH stimulation have also been reported in other forms of adrenal hyperplasia.

TEST PRINCIPLE

17OH Progesterone (antigen) in the sample competes with the antigenic 17OH Progesterone conjugated with horseradish peroxidase (HRP) for binding to the limited number of antibodies anti 17OH Progesterone coated on the microplate (solid phase).

After incubation, the bound/free separation is performed by a simple solid-phase washing. Then the enzyme HRP in the bound-fraction reacts with the Substrate (H_2O_2) and the TMB Substrate and develops a blue color that changes into yellow when the Stop Solution (H_2SO_4) is added. The color intensity is inversely proportional to the 17OH Progesterone concentration in the sample.

17OH Progesterone concentration in the sample is calculated through a calibration curve.

Reagent Preparation

1. Preparation of the Standard (S_0,S_1,S_2,S_3,S_4,S_5) and Control

The standard has the following concentration of 17α OH Progesterone:

	S_0	S_1	S_2	S_3	S_4	S_5
ng/ml	0	0.2	0.6	2	6	20

Stability: until the expiration date printed on the kit.
When are open, the standards are stable six months at +2-8°C.

1.1. Preparation of the Sample

The determination of 17OH Progesterone can be performed in human plasma as well as in serum.

Store the sample at -20°C if the determination is not performed on the same day of the sample connection. Avoid repetitive freezing and thawing of samples.
The Control is ready to use.

1.2 Preparation of the Wash Solution

Dilute the content of each vial of the "10X Conc. Wash Solution" with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect the 1:10 dilution ratio. The diluted wash solution is stable for 30 days at 2-8°C.

In concentrated wash solution is possible to observe the presence of crystals; in this case mix at room temperature until the complete dissolution of crystals; for greater accuracy, dilute the whole bottle of concentrated wash solution to 500 mL, taking care to transfer completely the crystals, then mix until crystals are completely dissolved.

Diagnostic Automation/ Cortez Diagnostics, Inc.
21250 Califa St, Suite 102 and 116, Woodland Hills, CA 91367 USA Phone: 818-591-3030, Fax : 818-591-8383
Email: onestep@rapidtest.com Website: www.rapidtest.com

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. Coated Microplate (1 breakable microplate coated with anti 17 OH progesterone antibody.
2. TMB-substrate (1 vial,15 mL)
   H_2O_2.TMB 0.26g/L (avoid any skin contact)
3. Stop solution (1 vial) 15 mL)
   Sulphuric acid 0.15 mol/L (avoid any skin contact)
4. 17OH Progesterone Standards (6 vials,1 ml each)
   STD0
   STD1
   STD2
   STD3
   STD4
   STD5
5. Control (1 vial = 1 ml)
   Control concentration is Lot-specific and it is stated on Certificate of Analysis.
6. Conjugate (1 vial = 22 mL)
   17OH Progesterone-HRP conjugate
7. 10X Conc. Wash Solution (1flacone, 50 mL)
   0.2M phosphate buffer, pH 7.4

Materials required but not provided

1. Distilled water
2. Automatic Dispenser
3. Microplate Reader (450 nm , 620-630 nm)
4. Microplates Washer

Notes

Store all reagents between 2÷8C°in the dark.
Open the bag of reagent 1 (Coated Mciroplate) only when it is at room temperature and close immediately after use. Once opened, it is stable until the expiry date of the kit. Do not remove the adhesive sheets on the unutilized strips.

ASSAY PROCEDURE

1. **Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes.** At the end of the assay, store immediately the reagents at 2-8°C: avoid long exposure to room temperature.
2. Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
3. To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
4. As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (S_0-S_5), two for each Control, two for each sample, one for Blank.

Reagent	Standard	Sample / Control	Blank
Standard S_0-S_5	**25 µL**		
Sample/Control		**25 µL**	
Conjugate	**200 µL**	**200 µL**	
Incubate at 37°C for 1 hour. Remove the contents from each well; wash the wells 3 times with 300 µL of diluted Wash Solution. **Important note:** during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel. **Automatic washer**: if you use automated equipment, wash the wells at least 5 times.			
TMB Substrate	**100 µL**	**100 µL**	**100 µL**
Incubate at room temperature 22÷28°C for *15 minutes* in the dark.			
Stop solution	**100 µL**	**100 µL**	**100 µL**
Shake the microplate gently. Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

RESULTS

1. **Mean Absorbance**
Calculate the mean of the absorbance (Em) for each point of the standard curve (S0-S5) and of each sample.

2. **Standard Curve**
Plot the mean value of absorbance (Em) of the standards (S0-S5) against concentration. Draw the best-fit curve through the plotted points. (es: Four Parameter Logistic).

3. **Calculation of results**
Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in ng/mL.

REFERENCE VALUE

The serum or plasma 17α OH Progesterone reference values are:

WOMEN:	follicular phase	0.2 - 1.3 ng/mL
	luteinic phase	1.0 - 4.5 ng/mL
	menopause	0.2 - 0.9 ng/mL
MEN:		0.2 - 2.3 ng/mL
CHILDREN		0.2 - 0.9 ng/mL

Please pay attention to the fact that the determination of a range of expected values for a "normal" population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the Manufacturer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of 17OH Progesterone for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents.

Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the calibration curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

PERFORMANCE CHARACTERISTICS

Precision

1. Intra Assay Variation

Within run variation was determined by replicate (20x) the measurement of three different sera in one assay. The within assay variability is ≤ 8.2%.

2. Inter Assay Variation

Between run variation was determined by replicate (10x) the measurement of three different sera in different lots. The between assay variability is ≤ 13.8%.

3. Accuracy

The recovery test performed on three different samples, enriched with 6.6 - 3.3 - 1.65 - 0.83 - 0.41 ng/mL of 17OH Progesterone gave an average value (±SD) of 98.66% ± 5.99%.

In the diluition test three different samples were diluted 2, 4 and 8 times with Calibrator 0; the average value (±SD) obtained is 95.31% ± 4.88%.

4. Sensitivity

The lowest detectable concentration of 17OH progesterone that can be distinguished from the zero standards is 0.05 ng/ml at the 95 % confidence limit.

5. Specificity

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

17αOH Progesterone	100 %
11-Deoxycortisol	0.846 %
Progesterone	0.590 %
Pregnenolone	0.250 %
Testosterone	0.017 %
17b Estradiol	< 0.001 %
Aldosterone	< 0.001 %
Estriol	< 0.001 %
Estrone 3-sulfate	< 0.001 %
Spironolactone	< 0.001 %
Androstenedione	< 0.01 %
Androsterone	< 0.01 %
Corticosterone	< 0.01 %
Cortisol	< 0.01 %
Cortisone	< 0.01 %
DHEA	< 0.01 %
DHEA-S	< 0.01 %
DHT	< 0.01 %
Prednisolone	< 0.01 %
Prednisone	< 0.01 %
Cholesterol	< 0.01 %

6. Correlation

The DAI 17OH Progesterone ELISA was compared to another commercially available 17OH progesterone assay. 65 Serum samples were tested

The regression curve is:
Y = 1.11* x + 0.04
R^2 = 0.935

The new DAI 17OH Progesterone ELISA kit was compared to the previous DAI 17OH Progesterone ELISA kit. 65 serum samples were tested.

The regression curve is:
Y = 1.31*X – 0.49
R^2 = 0.90

PRECAUTIONS

1. Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
2. All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
3. Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
4. Do not interchange kit components from different lots. The expiry dates printed on the labels of the box and of the vials must be observed. Do not use any kit component beyond their expiry date.
5. If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
6. The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background. To improve the performance of the kit on ELISA automatic systems, it is recommended to increase the number of washes.
7. It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate.
8. Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
9. Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
10. Maximum precision is required for reconstitution and dispensation of the reagents.
11. Samples microbiologically contaminated, highly lipemeic or haemolysed should not be used in the assay.
12. Plate readers measure vertically. Do not touch the bottom of the wells.

WARNINGS

- This kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- All human source material used in the preparation of the reagents has been tested and found negative for antibody to HIV 1&2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the reagents should be handled in the same manner as potentially infectious material.
- Some reagents contain small amounts of Sodium Azide or Proclin 300^R as preservative. Avoid the contact with skin or mucosa.
- Sodium Azide may be toxic if ingested or absorbed through the skin or eyes; moreover it may react with lead or copper plumbing to form potentially explosive metal azides. If you use a sink to remove the reagents, allow scroll through large amounts of water to prevent azide build-up.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.

- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.
- This method allows the determination of 17OH Progesterone from 0.2 ng/mL to 20 ng/mL.
- The clinical significance of the determination of 17OH Progesterone can be invalidated if the patient was treated with cortisone or natural or syntetic steroids.

WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

REFERENCES

1. Wisdom, G.B. Clin. Chem. 22/8 1243 - 1255 (1976)
2. De Villa, G.O. et al. J.Clin. Endoc. Metob. 35,458 (1972)
3. Hubl, W., et al Endokrinologie, 1982, 79 (2), 165
4. Arakawa, H., et al Chem. Pharm. Bull. Tokyo 30 (8) 3036 (1982)
5. D. Riad - Fanny, et al Endocr. Reviews, 3 (4) 304 367 (1982)

ISO 13485 ISO 9001 **Diagnostic Automation/Cortez Diagnostics, Inc. 21250 Califa Street, Suite 102 and 116, Woodland Hills, California 91367 USA**	
Date Adopted	**2015-11-03**
REF 1292-17	**AccuDiag™- 17 α OH Progesterone ELISA**
EC REP	**CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands. www.cepartner4u.eu**
Revision Date: 2015-01-01	