CE

AccuDiag™
17 α OH Progesterone
ELISA Kit

Cat# 1292-17

IVD See external Label 2°C-8°C Σ 96 Tests

Test	17 α OH Prgesterone
Method	Enzyme Linked Immunosorbent Assay
Principle	Competitive Enzyme Immunoassay
Detection Range	0-19.2 ng /ml
Sample	50 µL serum / plasma
Total Time	~75 min.
Shelf Life	12 -14 Months from the manufacturing date
Specificity	100 %
Sensitivity	0.09 ng/mL

INTENDED USE

Competitive immunoenzymatic colorimetric method for quantitative determination of 17α OH Progesterone concentration in serum and plasma

SUMMARY AND EXPLANATION

Diagnostic Automation 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.

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

17α OH Progesterone (antigen) in the sample competes with horseradish peroxidase 17α OH Progesterone (enzyme-labeled antigen) for binding onto the limited number of anti- 17α OH Progesterone coated on the microplates (solid phase).

After incubation, the bound/free separation is performed by a simple solid-phase washing.
The enzyme substrate (H_2O_2) and the TMB-substrate (TMB) are added. After an appropriate time has elapsed for maximum color development, the enzyme reaction is stopped and the absorbances are determined.

17α OH Progesterone concentration in the sample is calculated based on a series by a set of standard.
The color intensity is inversely proportional to the 17α OH Progesterone concentration in the sample.

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	0.4	1.6	6.4	19.2

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.

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. Coated Microplate (1 microplate breakable)
Anti- 17α OH Progesterone IgG adsorbed on microplate
REF DA-P/1292-17

2. TMB-substrate (1 bottle) 15 mL REF DA-T/1292-17
H_2O_2.TMB 0.26g/L (avoid any skin contact)

3. Stop solution (1 bottle) 15 mL REF DA-S/1292-17
Sulphuric acid 0.15 mol/L (avoid any skin contact)

4. 17OH Progesterone Standards 6x (1 vial = 1 ml)

STD0 REF DAS0-1292-17
STD1 REF DAS1-1292-17

STD2 REF DAS2/1292-17
STD3 REF DAS3/1292-17
STD4 REF DAS4/1292-17
STD5 REF DAS5/1292-17

5. Control (1 vial = 1 ml) REF DACON/1292-17

6. Conjugate (1 bottle) 6mL REF DA-C/1292-17
17OH Progesterone-HRP conjugate

Materials required but not provided
1. Distilled water.
2. Microplate Reader.
3. Microplates Washer.

Notes

Store all reagents between 2÷8C°in the dark.
Open the bag of reagent 3 (Antibody) only when it is at room temperature and close immediately after use. Do not remove the adhesive sheets on the unused strips.

ASSAY PROCEDURE

- **Allow all reagents to reach room temperature (22-28°C).**
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- 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 (C_0-C_5), two for each Control, two for each sample, one for Blank.

Reagent	Standard	Sample	Blank
Standard S_0-S_5	**50 µL**		
Control	**50 µL**		
Sample		**50 µL**	
Conjugate	**50 µL**	**50 µL**	
Incubate at 37°C for *1 hour*. Remove the contents from each well; wash the wells with 300 µL of distilled water. Repeat the washing procedure by draining the water completely.			
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**
Read the absorbance (E) at 450 nm against Blank.			

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.

4. Range of Control:
Please refer to the range information from the COA for the given Lot number. The obtained value for the control should fall within the specified range.

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 determination (16x) of two different control sera in one assay. The within assay variability is 5.7%.

2. Inter Assay Variation
Between run variation was determined by replicate measurements of three different control sera in 2 different lots. The between assay variability is 9%.

3. Accuracy
The recovery of 1 – 2 – 4 ng/mL of 17OH progesterone added to a sample gave an average value (±SD) of 99.7% ± 3.4% with reference to the original concentrations.

4. Sensitivity
The lowest detectable concentration of 17OH progesterone that can be distinguished from the zero standards is 0.1 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:

17 αOH progesterone	100%
17 αOH prognenolone	1.3 %
Progesterone	1.2 %
Cortisol	$2x10^{-2}$ %
Cholesterol	$8x10^{-4}$ %

6. Correlation with RIA
The DAI 17OH Progesterone ELISA was compared to another commercially available 17OH progesterone assay. Serum samples of 14 females, 8 children and 14 males were analysed according in both test systems.
y = 0.857 x + 0.06
r = 0.993 (r^2 = 0.986)

7. Hook Effect
The 17OH progesterone ELISA, a competitive enzyme immunoassay, shows no Hook Effect up to
40.5 ng/ml.

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.
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.
- Some reagents contain small amounts of Proclin 300^{R} as preservative. Avoid the contact with skin or mucosa.
- 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 19.2 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

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ISO 13485
ISO 9001

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EC REP	CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands. www.cepartner4u.eu

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