



DIAGNOSTIC AUTOMATION, INC.

23961 Craftsman Rd, Suite D/E/F, Calabasas, CA 91302

Tel: (818) 591-3030, Fax: (818) 591-8383

onestep@rapidtest.com

technicalsupport@rapidtest.com

www.rapidtest.com

IVD



See external label

2°C-8°C

Σ= 96tests

REF

Cat #1292Z

17 α OH Progesterone

Cat # 1292Z

Direct immunoenzymatic determination of 17 α OH Progesterone in serum or plasma.

For In Vitro Diagnostic Use

Cat # Number	1292Z
Test	17 α OH Progesterone
Method	Enzyme Linked Immunosorbent Assay
Principle	Peroxidase – Conjugated Competitive ELISA
Detection Range	0-19.2 ng/mL
Sample	20ul serum
Specificity	100%
Sensitivity	0.1ng/mL
Total Time	~ 110 min
Shelf Life	12-14 months

** Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account.*

INTENDED USE

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

CLINICAL SIGNIFICANCE

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 3 β -hydroxysteroid dehydrogenase/ Δ^{5-4} isomerase.

17 α -OHP has no defined physiologic role except as a precursor molecule.

Serum 17 α -OHP levels are age-dependent, with peak levels observed during fetal life and the immediate postnatal period. During the first week of life, serum 17 α -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 17 α -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 17 α -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 11 β -hydroxylase, lead to a build-up of 17OHP. In contrast, the rare patient with 17 α -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.

PRINCIPLE

17 α OH Progesterone (antigen) in the sample competes with horseradish peroxidase 17 α OH Progesterone (enzyme-labelled antigen) for binding onto the limited number of anti- 17 α 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 (H₂O₂) and the TMB-substrate (TMB) are added. After an appropriate time has elapsed for maximum colour development, the enzyme reaction is stopped and the absorbances are determined.

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

REAGENT, MATERIAL AND INSTRUMENTATION

- 1. Coated Microplate (1 microplate breakable)**
Anti- 17 α OH Progesterone IgG adsorbed on microplate **REF DA-P/1292Z**
- 2. TMB-substrate (1 bottle) 15 mL**
H₂O₂.TMB 0.25gr/L (avoid any skin contact) **REF DA-T/1292Z**
- 3. Stop solution (1 bottle) 15 mL**
Sulphuric acid 0.15 mol/L (avoid any skin contact)
Reagent and material supplied in the kit **REF DA-S/1292Z**
- 4. 17OH Progesterone Standards 6x (1 vial = 1 ml)**

STD0	REF DAS0-1292Z
STD1	REF DAS1-1292Z
STD2	REF DAS2/1292Z
STD3	REF DAS3/1292Z
STD4	REF DAS4/1292Z
STD5	REF DAS5/1292Z
- 5. Contol (1 vial = 1 ml)** **REF DACON/1292Z**
- 6. Conjugate (1 bottle) 6mL** **REF DA-C/1292Z**
17OH Progesterone-HRP conjugate

Reagents necessary not supplied in the kit
Distilled water

Auxiliary materials and instrumentation

Microplate Reader (Cat # DAR800)
Microplate Washer (Cat # DAW50)

Notes

Store all reagents between +2 and + 8C°in the dark.

Open the bag of reagent 3 (COATED MICROPLATE) only when it is at room temperature and close immediately after use.

Do not remove the adesive sheets on the unused strips

PRECAUTION

- Do not use heavily hemolized samples.
- Do not use differents lots of reagents.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Avoid the exposure of reagent TMB/H₂O₂ to directed sunlight, metals or oxidants.
- This method allows the determenation of 17 α OH Progesterone from 0.2 ng/mL to 19.2 ng/mL.
- The clinical significance of the determenation of 17 α OH Progesterone can be invalidated if the patient was treated with cortisone or natural or syntetic steroids.
- The reagent contain Proclin 300R as perservative

PROCEDURE

1. Preparation of the Standard (S₀,S₁,S₂,S₃,S₄,S₅) and Control

The standard has the following concentration of 17 α 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 +4°C.

2. PROCEDURE

As it is necessary to perform the determination in duplicate, prepare two wells for each of the six points of the standard curve (S₀-S₅), and 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	
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.			

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 standard 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.

LIMITATION OF PROCEDURE

1. Assay Performance.

Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipemic or haemolysed specimen(s) should similarly not be used. 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 (10) minutes to avoid assay drift. If more than one (1) plate is used, it is recommended to repeat the dose response curve. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the

addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction. Plate readers measure vertically. Do not touch the bottom of the wells. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.

2. Interpretation.

If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

RESULTS

1. Mean Absorbance

Calculate the mean of the absorbance (Em) for each point of the standard curve and of each sample

2. Standard curve

Plot the mean value of absorbance of the standards (Em) 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 17 α OH Progesterone reference values are:

WOMAN:	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

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 (\pm SD) of 99.7% \pm 3.4% with reference to the original concentrations.

4. Specificity

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

17 α OH progesterone	100 %
17 α OH progrenolone	1.3 %
Progesterone	1.2 %
Cortisol	2×10^{-2} %
Cholesterol	8×10^{-4} %

5. 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.

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.

WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

REFERENCE

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)

Date Adopted	Reference No.
2011-06-30	DA-17 OH-Progesterone -2011



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