






23961 Craftsman Rd, Suite E/F
Calabasas, CA 91302
Tel: (818) 591-3030, Fax: (818) 591-8383
Email: onestep@rapidtest.com
Website: www.rapidtest.com

17 alpha OH Progesterone Microwell Serum ELISA For In Vitro Diagnostic Use

IVD  See external label  2°C-8°C  Σ= tests **REF** cat. #1292-6

INTRODUCTION

The steroid 17- α -Hydroxyprogesterone (17- α -OHP) is produced by both the adrenal cortex and gonads. Even though 17- α -OHP has relatively little progestational activity, it is of intense clinical interest because it is the immediate precursor to 11-desoxycortisol (Cpd-S). Because Cpd-S is produced by 21-hydroxylation of 17- α -OHP, measurement of 17- α -OHP is a useful indirect indicator of 21-hydroxylase activity. In congenital 21-hydroxylase deficiency, the most common variety of congenital adrenal hyperplasia (CAH), 17- α -OHP is secreted in abundant excess. It is moderately elevated in the 11- β -hydroxylase deficiency as well. Measurement of 17- α -OHP is therefore valuable in the initial diagnosis of CAH.

CLINICAL PHYSIOLOGY

A. Adult non-pregnant women:

In adult non-pregnant women in the childbearing age group, 17- α -OHP concentrations vary over the menstrual cycle with luteal phase concentrations being higher than follicular phase concentrations. This is because 17- α -OHP is secreted parallel with progesterone from maturing follicles or from the corpus luteum. There is also a diurnal variation of 17- α -OHP concentrations. This rhythm is parallel with adrenal cortisol secretion such that maximum 17- α -OHP concentrations are measured in samples obtained between midnight and 8:00 am.

B. Adult males:

There is little information available on the systematic variability of 17- α -OHP concentration in adult males.

C. Pregnant women and newborn children:

The steroid 17- α -OHP is produced in large amounts by the fetus and the adrenals. It is secreted in abundance into both the fetal and maternal circulation. The maternal concentrations of 17- α -OHP increase very sharply after 32 weeks gestational age to about 4-fold above basal concentrations at term.

CLINICAL APPLICATIONS

A. Congenital adrenal hyperplasia:

The principal application of the 17- α -OHP RIA is in the diagnosis of CAH in newborns with ambiguous genitalia and in virilized adolescent girls. Since 17- α -OHP is the immediate precursor to 11-desoxycortisol, basal 17- α -OHP concentrations are sharply elevated in patients with 21-hydroxylase deficiency and to a lesser degree in patients with 11-hydroxylase deficiency. Because 17- α -OHP concentrations are so markedly elevated in newborns and adolescent girls afflicted with CAH, a single basal measurement is all that is normally required to make the diagnosis.

B. Late onset adrenal hyperplasia:

More recently, 17- α -OHP concentrations have been utilized in the evaluation of androgenized women where late onset 21-hydroxylase is suspected. This condition is clinically very subtle and since the presentation is the same as classical polycystic ovarian

disease, basal plasma 17- α -OHP concentrations, unlike classical congenital adrenal hyperplasia, are normal. The diagnosis is made by administration of an ACTH stimulation test.

C. Other applications:

Measurement of 17- α -OHP concentrations is also utilized in evaluation of both men and women with acne vulgaris, male pattern baldness and in some subtle forms of infertility. Experience with these applications are very limited.

PRINCIPLE

The **DIAGNOSTIC AUTOMATION, INC. 17- α -OH Progesterone ELISA KIT** is based on the competition principle and the microplate separation. An unknown amount of 17- α -OHP present in the sample and a fixed amount of 17- α -OHP conjugated with horse-radish peroxidase compete for the binding sites of a polyclonal 17- α -OHP antiserum coated onto the wells. After one hour incubation the microtiterplate is washed to stop the competition reaction. Having added the substrate solution the concentration of 17- α -OHP is inversely proportional to the optical density measured.

REAGENTS

1. *Microtiter wells* coated with Anti-17- α -OH Progesterone serum (96 wells).
2. *Enzyme-Conjugate*, 25 ml
17- α -OH Progesterone conjugated to horseradish peroxidase.
3. *Reference Standard Set*, 1 ml each
0,15; 0,5; 1,5; 3; 7,5; 20 ng/ml.
4. *Zero Standard* or *Specimen Diluent*, 1 ml.
5. *Substrate Solution TMB*, 25 ml.
6. *Stop Solution*, 0,5M H₂SO₄, 14 ml.
7. *Wash Solution*, 40X, 30 ml.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. A microtiterplate reader (450 \pm 10 nm)(e.g. the DIAGNOSTIC AUTOMATION, INC. Instruments Microtiterplate Reader).
2. Precision micropipettes with disposable tips for 100 and 200 μ l.
3. Standard refrigerator.
4. Absorbent paper.
5. Deionized water.

STORAGE CONDITIONS

When stored at 2° to 8°C unbroken reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Enzyme-Conjugate, Standard Solution, Substrate Solution, Wash Solution and *Zero Standard* must be stored at 2° to 8°C.

Microtiter wells must be stored at 2° to 8°C. Once the foil bag has been broken, care should be taken to close it tightly again. The immuno-reactivity of the coated microtiter wells is stable for approx. 6 weeks in the broken, but tightly closed bag containing the desiccant.

WARNINGS AND PRECAUTIONS FOR USERS

1. **CAUTION:** Test methods are not available which can offer complete assurance that Hepatitis B virus, Human Immunodeficiency Virus (HIV/HTLV-III/LAV), or other infectious agents are absent from the reagents in this kit. Therefore, all human blood products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation, where it exists (e.g., USA Center for Disease Control/National Institute of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories," 1984).
2. Avoid contact with *Stop Solution*, 0,5M H₂SO₄. It may cause skin irritation and burns.
3. Replace caps on reagents immediately. Do not switch caps.
4. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
5. Do not pipette reagents by mouth.
6. For in vitro diagnostic use only.
7. Do not mix or use components from kits with different lot numbers.

SPECIMEN COLLECTION AND PREPARATION

1. Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature. No special pretreatment of sample is necessary. The specimen may be stored at 2-8° C for up to 24 hours, and should be frozen at -10° C or lower for longer periods. Do not use grossly hemolyzed or grossly lipemic specimens.
2. Samples suspected to contain 17- α -OH Progesterone concentration higher than 20 ng/ml are to be diluted with *Zero Standard*.

Please note: Samples containing sodium azide should not be used in the assay.

PERFORMANCE OF THE ASSAY

GENERAL REMARKS:

1. All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
2. Once the test has been started, all steps should be completed without interruption.
3. Use new disposable plastic pipette tips for each reagent, standard or specimen in order to avoid cross contamination. For the dispensing of the Substrate Solution and the Stop Solution avoid pipettes with metal parts.
4. Pipette standards and samples onto the bottom of the well. For pipetting of Enzyme Conjugate and Stop Solution it is recommended to hold the pipette in a vertical position above the well and dispense the correspondent solution into the centre of the well so that a complete mixing of Enzyme Conjugate with sample or standard and of the Stop Solution with the Substrate Solution is achieved.
5. Before starting the assay, it is recommended that all reagents be ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
6. As a general rule the enzymatic reaction is linearly proportional to time and temperature. This makes interpolation possible for fixed physico-chemical conditions. If in a test run the absorbance of Zero Standard is lower than 1,0 or above the upper performance limit of your microtiterplate spectrophotometer you can extend or reduce the incubation time of the final enzymatic formation of color to 45 or 15 minutes accordingly. Since calibrators are assayed in each run, absorbance fluctuations do not affect the result.
7. The Substrate Solution should be colourless or slightly blue or green. If the solution is dark blue the reagent is unusable and must be discarded.
8. During incubation with Substrate Solution avoid direct sunlight on the microtiter plate.

REAGENT PREPARATION

Wash Solution: Add deionized water to the 40x concentrated Wash Solution (contents: 30 ml) to a final volume of 1200 ml. The diluted Wash Solution is stable for 2 weeks at room temperature.

ASSAY PROCEDURE

1. Secure the desired number of coated strips in the holder.
2. Dispense 25 μ l of 17- α -OH Progesterone *Standards* into appropriate wells.
3. Dispense 25 μ l of sample into selected wells. Time between distribution of first Standard and last sample can be up to 10 minutes without affecting the results.
4. Incubate plate for 5 minutes at room temperature.
5. Dispense 200 μ l of *Enzyme-Conjugate* into each well.
6. Thoroughly mix the plate for 10 seconds. It is important to have complete mixing in this step.
7. Incubate for 60 minutes at room temperature.
8. Briskly shake out the contents of the wells.
9. Rinse the wells 3 times with *diluted Wash Solution* (400 μ l per well). Strike the wells sharply on absorbent paper to remove residual droplets.
10. Add 200 μ l of *Substrate Solution* to each well, at timed intervals.
11. Incubate for 30 minutes at room temperature.
12. Stop the enzymatic reaction by adding 100 μ l of *Stop Solution* to each well at the same timed intervals as in step 10 and determine the absorbance of each well at 450 \pm 10 nm.

Final Reaction Stability

It is recommended that the wells be read within 10 minutes following step 12.

CALCULATION OF RESULTS

Any microwell reader capable of determining the absorbance at 450 \pm 10nm may be used. The 17- α -OH Progesterone value of each serum sample is obtained as follows :

- Using linear-linear or semi log graph paper, construct a standard curve by plotting the average absorbance (Y) of each Reference Standard against its corresponding concentration (X) in ng/ml. For construction of the standard curve we recommend a four parameter logistic function.
- Use the average absorbance of each serum sample to determine the corresponding 17- α -OH Progesterone value by simple interpolation from this standard curve, multiplying by the initial sample dilution, if necessary.
The DIAGNOSTIC AUTOMATION, INC. Regression Program allow the reading and computer assisted interpretation using a four parameter logistic function.

EXPECTED NORMAL VALUES

Newborn	5 - 30 day	< 0,7 - 2,5 ng/ml
	31 - 60 day m.	0,8 - 5,0 ng/ml
	f.	0,5 - 2,3 ng/ml
Children	3 - 14 years	0,07- 1,7 ng/ml

Reproductive aged women

Follicular phase:	0,1 - 0,8 ng/ml
Luteal phase:	0,6 - 2,3 ng/ml
Ovulation:	0,3 - 1,4 ng/ml
Post ACTH:	< 3,2 ng/ml
Third trimester:	2,0 - 12 ng/ml
Postmenopausal women	0,13 - 0,51 ng/ml
Normal men	0,5 - 2,1 ng/ml

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

Standard	Optical Units
Standard 0 (0 ng/ml)	1,92
Standard 1 (0,15 ng/ml)	1,53
Standard 2 (0,5 ng/ml)	1,17
Standard 3 (1,5 ng/ml)	0,85
Standard 4 (3,0 ng/ml)	0,60
Standard 5 (7,5 ng/ml)	0,35
Standard 6 (20 ng/ml)	0,17

Option II: Determination of 17- α -OH Progesterone in Saliva

Sample Preparation

We recommend to freeze the saliva samples at -20° C immediately after sample collection.

Before testing thaw the samples, mix by vortexing and then centrifuge the saliva samples. The supernatant is used for hormone analysis.

Extraction for the determination of 17- α -OH Progesterone in Saliva:

The saliva samples have to be extracted. Although the standards have been prepared in Zero Standard and the saliva extracts of the saliva samples are reconstituted with Zero Standard, too, we recommend to extract the standards as well, to avoid extraction mistakes. The following extraction method is recommended:

- Pipet 50 μ l Control-/ Standard serum (Test kit) or 250 μ l Saliva Samples in tightly closed glass tubes (Volume approx. 3 ml).
- Add 1 ml anaesthetic ether (Hoechst AG, Germany) into each tube.
- Vortex 1 h, then freeze 1 h (or more) at -20° C or lower.
- Decant the ether phase in a new glass tube and evaporate (this takes ca. 2 hours in an exsikkator). After evaporation the tubes should visually look empty.
- Reconstitute the extract with 250 μ l Zero Standard of the test kit.
- Vortex 15 Min.
- Continue as described in the instruction manual (see Assay Procedure, p.4).

Please note: When using saliva samples the patient results have to be divided by 5 (since you have used the 5-fold volume).

PERFORMANCE CHARACTERISTICS

Sensitivity

The lowest detectable level of 17- α -OH Progesterone that can be distinguished from the Zero Standard is 0,05 ng/ml at the 95 % confidence limit.

Specificity

The following materials have been checked for cross reactivity. The percentage indicate cross reactivity at 50% displacement compared to 17-OH Progesterone.

Steroid	% Cross Reaction
17- α -OH Progesterone	100,0
Estriol	< 0,01
Estradiol 17 β	< 0,01
Testosterone	< 0,01
Dihydrotestosterone	< 0,01
DOC	0,05
11-Desoxicortisol	1,4
Progesterone	1,2
DHEA	< 0,01
DHEAS	< 0,001
Cortisol	< 0,01
Corticosterone	< 0,05
Aldosterone	< 0,01
Androstendione	< 0,01
Dehydroepiandrosten sulfate	< 0,01
Prednison	< 0,01

Precision

Serum	Intraassay			Interassay		
	n	<X> \pm SD ng/ml	CV %	n	<X> \pm SD ng/ml	CV %
1	10	0,99 \pm 0,08	8,1	10	0,95 \pm 0,09	9,5
2	10	2,34 \pm 0,10	4,3	10	2,24 \pm 0,18	8,0
3	10	6,75 \pm 0,34	5,0	10	6,52 \pm 0,49	7,5

Accuracy

The accuracy of the assay was evaluated by recovery and dilution tests.

Recovery test

Serum	Endogenous 17- α -OHP ng/ml	Added 17- α -OHP ng/ml	Recovery %
1	3,1	5	105
		2	103
		1	97
2	1,9	5	102
		2	105
		1	99
		0,3	101

Dilution test

Serum	Dilution factor	Measured conc. ng/ml	Recovery %
1	Undiluted	1,23	
	1:2	0,62	101
	1:4	0,29	94
	1:8	0,15	98
2	Undiluted	6,21	
	1:2	2,93	94
	1:4	1,62	104
	1:8	0,76	98
	1:16	0,37	95
1:32	0,17	88	
3	Undiluted	7,35	
	1:2	3,72	101
	1:4	1,91	104
	1:8	0,93	101
	1:16	0,44	96
1:32	0,24	104	

QUALITY CONTROL

Good laboratory practice requires that controls are run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

We recommend to use BIO RAD Lyphochek Immunoassay Control Sera which are also available from DIAGNOSTIC AUTOMATION, INC..

LIMITATION OF PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instruction and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbances.
3. Complete mixing of Conjugate with standard or sample (step 5) and of Stop Solution with Substrate Solution (step 12) is critical. Insufficient mixing will result in poor precision.

REFERENCES

1. Abraham, G.E., R.S. Swerdloff, D. Tulchinsky et al: Radioimmunoassay of plasma 17- hydroxyprogesterone. J. Clin. Endocrinol. Metab. 33:42, 1971
2. Chrousos, G.P., D. L. Loriaux, D.L. Mann, et al: Late onset 21- hydroxylase deficiency mimicking idiopathic hirsutism or polycystic ovarian disease. Annals Intern. Med. 96:143, 1982.
3. Buster, J.E., R.J. Chang, D.L. Preston, et al: Interrelationships of circulating maternal steroids; progesterone, 16a-hydroxyprogesterone, 17a-hydroxyprogesterone, 20a-dihydroprogesterone, gamma-5-pregnenolone, gamma-5- pregnolone-sulfate, gamma-5-pregnenolone-sulfate and 17-hydroxy gamma-5-pregnenolone, J. Clin. Endocrinol. Metab. 48:133, 1979.
4. New, M.I., B. Dupont, S. Pang, et al: An update on congenital adrenal hyperplasia. Recent Progress in Hormone Research, 37:105, 1981. 5. Pang S., J. Hotchkiss, A. Drash, et al: Micro filter paper method for 17a-hydroxyprogesterone radioimmunoassay: Its application for rapid screening for congenital adrenal hyperplasia. J. Clin. Endocrin. Metab., 45:1003, 1977.
6. Lobo, R.A., U. Goebelsmann: Adult manifestation of congenital adrenal hyperplasia due to incomplete 21-hydroxylase deficiency mimicking polycystic ovarian disease. Am. J. Obstet. Gynecol., 138:720, 1980.
7. Urban, M.D., P.A. Lee and C.J. Migeon: Adult high infertility in men with congenital adrenalized hyperplasia. N. Engl. J. Med. 299:1392, 1978.
8. Meikle, A.W., R.J. Worley and C.D. West: Adrenal corticoid hyper-response in hirsute women. Fertil. Steril. 41:575, 1984



DIAGNOSTIC AUTOMATION, INC.

23961 Craftsman Road, Suite E/F, Calabasas, CA 91302

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

ISO 13485-2003

PROCEDURE FLOW SHEET DIAGNOSTIC AUTOMATION, INC. 17- α -OH PROGESTERONE ELISA KIT

Description	Standard Sample μ l		Enzyme-Conjugate μ l		Substrate Solution μ l		Stop Solution μ l		Results ng/ml
Standard 0	25	Incubate for 5 minutes at room temperature.	200	Mix for	200	Incubate for	100	Read the OD at 450 nm with a microtiter-plate reader.	0
Standard 1	25		200	10 seconds.	200	30 minutes	100		0,15
Standard 2	25		200	Incubate for	200	at room	100		0,5
Standard 3	25		200	60 minutes	200	temperature	100		1,5
Standard 4	25		200	at room	200		100		3
Standard 5	25		200	temperature.	200		100		7,5
Standard 6	25		200	Rinse the	200		100	20	
Sample 1	25		200	wells 3 times	200		100		
Sample 2	25		200	with diluted	200		100		
Sample 3	25		200	<i>Wash Solution</i>	200		100		
Sample 4	25		200	400 μ l/well	200		100		

REVISION DATE: 3/27/06