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17-a-OH-Progesterone

(en) English

REF **K00192**

Content

-1 Microwell Plate: 96 wells (12 x 8 well antibody coated strips, individual breakaway)
-1x 22 mL Enzyme Conjugate
-1x 50 mL Wash Buffer
-1x 15 mL Substrate Solution
-1x 15 mL Stop Solution
-6x 1 mL Calibrators
-2x 1 mL Controls
-1 Package Insert
-1 Certificate of Analysis

For professional in vitro diagnostic use only.

INTENDED USE

17OH Progesterone ELISA is a manual *in vitro* diagnostic device intended for the quantitative determination of 17OH Progesterone in human serum or plasma from adult and paediatric populations. Results are to be used in conjunction with other clinical and laboratory data as an aid in the diagnosis and monitoring of various disorders of the adrenal glands.
17-a-OH-Progesterone ELISA kit is intended for laboratory use only.

DIAGNOSTIC SIGNIFICANCE

17-α-OH Progesterone (17-OH P) is an important intermediate of steroid biosynthesis. Elevated levels of 17-OH P are linked to dysfunction of steroid biosynthetic pathway that leads to an androgen excess. Therefore, measurements of 17-OH P are useful for the differential diagnosis of clinical conditions related to an hyperandrogenic phenotype and linked to adrenal disfunctions in both sexes.
Congenital Adrenal Hyperplasia (CAH) is a group of autosomal recessive disorders characterised by impaired cortisol synthesis[1-4]. The predominant cause of the condition (95% of cases) is mutations of the *CYP21A2* gene that encodes the adrenal steroid 21-hydroxylase. 21-hydroxylase is responsible for conversion of 17-OHP to 1-deoxycortisol and progesterone to deoxycorticosterone. Assessment of 17-OH P levels provides supporting information for the differential diagnosis of CAH caused by mutations of the genes involved in cortisol synthesis from CYP21A2 gene mutations.
Measurement of 17-OH P is also considered useful in supporting diagnosis of other conditions characterised by hyperandrogenism such as polycystic ovarian syndrome (PCOS)[5] and hirsutism in women, precocious puberty[6] and precocious adrenarche. Determination of 17-OH P levels are used in such cases to exclude a diagnosis of CAH and non-classical CAH.
Patients affected by CAH shall be treated with glucocorticoids and mineral-corticoids. Treatment with glucocorticoids is of particular importance to avoid adrenal crisis and virilization due to excessive androgen synthesis[2,4,7]. Since the goal of glucocorticoid treatments is not the complete suppression of 17-OH P synthesis, it is important to ensure appropriate monitoring of treatment to evaluate the correct dosage of the drugs. This is managed by the regular assessment of 17-OH P (and androstenedione) levels as traditional indicators of the adequacy of glucocorticoids treatment in CAH[5].

TEST PRINCIPLE

The 17-a-OH-Progesterone ELISA is a competitive enzyme immunometric assay where 17-α-OH Progesterone (antigen) in the sample competes with the antigenic 17-α-OH Progesterone conjugated with horseradish peroxidase (HRP) for binding to the limited number of antibodies anti 17-α-OH 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 Substrate Solution (TMB) and develops a blue colour that changes into yellow when the Stop Solution (H_2SO_4) is added. The colour intensity is inversely proportional to the 17-α-OH Progesterone concentration in the sample.
17-α-OH Progesterone concentration in the sample is calculated through a calibration curve.

REAGENT COMPOSITION

Calibrators **C_A-C_B-C_C-C_D-C_E-C_F**	Calibrators contain the following concentrations of 17-α-OH Progesterone: C_A C_B C_C C_D C_E C_F

	ng/mL 0 0.2 0.6 2.0 6.0 16.0 Human serum, ProClin >0.0015%, NaN_3 <0.1%
Controls A / B	17-α-OH Progesterone concentration of the controls is lot-specific is indicated on the Certificate of Analysis Human serum, ProClin >0.0015%, NaN_3 <0.1%
Enzyme Conjugate	17-α-OH Progesterone conjugated with Horseradish peroxidase (HRP) ProClin >0.0015%, BSA 0.1%
Microwell Plate	Microplate coated with anti-17-α-OH Progesterone antibody
Substrate Solution	H_2O_2-TMB 0.26 g/L (*avoid any skin contact!)* ProClin <0.0015%
Stop Solution	Sulphuric acid 0.15 mol/L (*avoid any skin contact!)*
Wash Buffer (10x)	0.2 M phosphate buffer pH 7.4; 10x concentrated ProClin >0.0015%

MATERIAL REQUIRED BUT NOT PROVIDED

- Distilled water
- Automatic dispenser
- Precision pipetting devices
- Microplate reader (450 nm, 620-630 nm)

REAGENT PREPARATION

Preparation Calibrators and Controls
The Calibrators and Controls are ready to use.
The Calibrators have the following concentration of 17-α-OH Progesterone:

	C_A	C_B	C_C	C_D	C_E	C_F
ng/mL	0	0.2	0.6	2	6	16

Preparation of the Conjugate
The conjugate is ready to use.

Preparation of the Wash Buffer
Dilute the content of each vial of the "Wash Buffer (10x)" 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 the concentrated Wash Buffer 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.

STORAGE AND STABILITY

Store the kit and all reagents at 2-8°C in the dark. The kit is stable up to the expiry date stated on the label and on the Certificate of Analysis.
Avoid using the kit or its components after the expiry date.
Open the bag of the microtiter plate only when it is at room temperature and close it immediately after use. Do not remove the adhesive sheet on the unutilised strips.
Once opened the kit is stable for 6 months at 2-8°C. For storage of samples please refer to section “Specimen Collection and Storage”.

WARNINGS AND PRECAUTIONS

- 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.
- ⚠ Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine protein has been obtained from BSE free countries, but these materials should be handled as potentially infectious.
- ⚠ 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 (calibrators, controls, conjugate and wash solution) contain small amounts of Sodium Azide or Proclin 300 (>0.0015%, <0.06%) as preservative. Avoid the contact with skin or mucosa.

- **Classification according to Regulation (EC) No. 1272/2008 [CLP]**
  Skin sensitivity, Category 1

Contains: ProClin 300

Warning

Hazard statements:
H317 - May cause an allergic skin reaction.

Precautionary statements:
P261 - Avoid breathing dust/fume/gas/mist/vapours/spray.
P280 - Wear protective gloves/protective clothing/eye protection/face protection/hearing protection.
P321 - Specific treatment (see supplemental first aid instruction on this label).
P333+P313 - If skin irritation or rash occurs: Get medical advice/attention.
P362+P364 - Take off contaminated clothing and wash it before reuse.

- Some reagents (calibrators and controls) contain small amounts of Sodium Azide (NaN_3) <0.1% as preservative 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, wash through with large amounts of water to prevent azide build-up.
- The Substrate Solution (TMB) 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 the Substrate Solution to direct sunlight, metals, or oxidants. Do not freeze the solution.

- 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.
- 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.
- Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately validated for its intended use/purpose.
- 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 automatic systems is recommended to increase the number of washes.
- 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.
- Addition of the Substrate Solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the Substrate Solution and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of reagents.
- Samples microbiologically contaminated, highly lipemic, icteric or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Fresh disposable tips must be used when pipetting assay reagents including samples, calibrators and controls to mitigate the risk of carryover contamination. Failure to do so may lead to invalid results.

SPECIMEN COLLECTION AND STORAGE

The assay should be performed using serum (standard sampling tubes or tubes containing serum separating gel) or plasma (lithium heparin, sodium heparin or potassium EDTA) samples.
If the assay is not performed on the same day of the sample collection, the samples can be stored at 2-8°C for 4 days. The samples can withstand 4 freeze-thaw cycles, but it is recommended to limit these cycles, and in the case, it is recommended to aliquot the samples.

Store the sample at -20°C if the determination is not performed on the same day of the sample collection. Before using, mix gently, for 5 minutes, with a roller mixer.

TEST PROCEDURE

- 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.
- 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_A-C_F), two for each Control, two for each sample, one for Blank.

Reagent	**Calibrator**	**Sample / Control**	**Blank**
Calibrator C_A – C_F	**25 µL**		
Sample / Control		**25 µL**	
Enzyme Conjugate	**200 µL**	**200 µL**	
Incubate at 37°C (± 0.5 ºC) for 1 hour. Remove the content from each well; wash the wells 6 times with 300 µL of diluted Wash Buffer. **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. In the case you use an automatic washer, after the last washing cycle remove the excess solution at the same manner.			
Substrate Solution	**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.			

INTERPRETATION OF RESULTS

A variety of data reduction software packages are available, which may be employed to generate the mean calibration curve and to calculate the mean concentrations of unknown samples and controls. A 4-parameter logistic (4PL) curve fit, **including Calibrator 0 is required.**

Alternatively, a calibration curve may be prepared on semi-log graph paper by plotting mean absorbance on the Y-axis against concentration of analyte on the X-axis. Calibrator 0 should be included in the calibration curve. Read the mean absorbance value of each unknown sample off the curve.

In order for the assay results to be considered valid the kit calibrators and control must fall within the specifications detailed in the lot specific certificate of analysis.

If a control is out of its specified range, the associated test results are invalid and samples must be retested.

QUALITY CONTROL AND CALIBRATION

Quality Control:
Good Laboratory Practice (GLP) requires the use of quality control specimens in each series of assays in order to check the performance of the assay. Controls should be treated as unknown samples, and the results analysed with appropriate statistical methods.
The kit controls provided in the kit should be tested as unknowns and are intended to assist in assessing the validity of results obtained with each assay plate.
The mean concentration of each control level is documented in the QC report included with each kit. These mean concentration levels are determined over several assays which are run in duplicate in multiple locations across each plate.
Dialab recommends the users to maintain graphic records of the control values generated with each assay run, including the running means, SDs and %CVs. This information will facilitate the controls trending analysis relating to

the performance of current and historical control lots relative to the supplied Quality Control data. The trending will assist in the identification of assays which give control values significantly different from their average range.
When interpreting control data, users should note that this product was designed and developed as a manual product. The range stated on the QC certificate should be appropriate for assays that are performed manually and with strict adherence to the Assay Procedure described above. It is recognised by Quality Control professionals, that as a result of differences in conditions and practices, there will always be variability in the mean values and precision of control measurements between different laboratories[8].

PERFORMANCE CHARACTERISTICS

Representative performance data are shown. Results obtained at individual laboratories may vary.

- **Measuring range**
  The assay measuring range (AMR) is 0.26 – 16 ng/mL. Any value that reads below 0.26 ng/mL should be reported as "< 0.26 ng/mL". Any value that reads above 16 ng/mL should be reported as "> 16 ng/mL".

- **Analytical sensitivity**
  The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) were determined with guidance from CLSI EP17-A, "Protocols for Determination of Limits of Detection and Limits of Quantitation" using 6 blanks and 6 low level samples.

	Results [ng/mL]
LoB	0.02
LoD	0.11
LoQ	0.26

- **Trueness**
  Trueness has been demonstrated through method comparison of the 17-a-OH-Progesterone ELISA to a commercially available LC-MS/MS using native donor samples. See subsection "Method Comparison"

- **Precision**
  Precision of the Dialab 17-a-OH-Progesterone ELISA was determined by performing a complex precision study.

  Repeatability
  A total of 6 serum samples were assayed in 5 replicates, once a day for 5 days by 3 operators. Data from one representative lot is shown below:

Sample	**n**	**Mean (ng/mL]**	**SD**	**CV%**
1	75	0.90	0.10	11.4%
2	75	1.45	0.15	10.0%
3	75	2.10	0.20	9.3%
4	75	5.14	0.49	9.5%
5	75	8.94	0.74	8.3%
6	75	14.50	1.11	7.6%

  Reproducibility
  total of 6 serum samples were assayed in 5 replicates, once a day for 5 days by 3 operators. Data from one representative lot is shown below:

Sample	**n**	**Mean [ng/mL]**	**SD**	**CV%**
1	75	0.90	0.12	13.0%
2	75	1.45	0.17	11.6%
3	75	2.10	0.23	11.1%
4	75	5.14	0.55	10.7%
5	75	8.94	0.82	9.2%
6	75	14.50	1.29	8.9%

- **Linearity**
  Linearity was evaluated based on CLSI EP-06, "Evaluation of the Linearity of Quantitative Measurement Procedures". For 17-a-OH-Progesterone concentration by 17-a-OH-Progesterone ELISA, the measurement procedure shows linearity for the interval from 0.2 to 17.22 ng/mL within the allowable deviation of linearity (ADL) of ±15 %.

- **Method comparison**
  The 17-a-OH-Progesterone ELISA was compared to a commercially available LC-MS/MS method following CLSI EP-9A, "Method Comparison and Bias Estimation Using Patient Samples". A total of 120 samples, selected to represent a wide range of 17-a-OH-Progesterone concentrations was assayed by each method. Passing-Bablok regression analysis was performed on the comparative data:

n	Slope (95% CI)	Intercept [ng/mL] (95% CI)	Correlation coefficient (r)
120	0.91 (0.87-0.97)	0.21 (0.16 – 0.28)	0.99

- **Analytical specificity**
  The specificity was assessed with the following cross-reactants:

Cross-reactant	Concentration tested	Mean %Cross reactivity
11-deoxycortisol	80 ng/mL	2.15%
Progesterone	2000 ng/L	0.47%
Pregnenolone	2000 ng/mL	0.15%
Testosterone	2000 ng/mL	0.07%
17β-Estradiol	2000 ng/mL	0.01%
Aldosterone	2000 ng/mL	0.00%
Estriol	2000 ng/mL	0.00%
Estrone-3-Sulphate	2000 ng/mL	0.00%
Spironolactone	2000 ng/mL	0.00%
Androstenedione	2000 ng/mL	0.05%
Androsterone	2000 ng/mL	0.04%
Corticosterone	2000 ng/mL	0.09%
Cortisol	2000 ng/mL	0.10%
Cortisone	2000 ng/mL	0.07%
DHEA	2000 ng/mL	0.07%
DHEA-S	20000 ng/mL	0.01%
DHT	2000 ng/mL	0.04%
Prednisolone	3000 ng/mL	0.02%
Prednisone	2000 ng/mL	0.01%

  The following substances do not interfere with a bias of >±15% in the 17-a-OH-Progesterone ELISA when the concentrations are below the stated threshold presented in the following table.

Potentially Interfering Reagent	Concentration tested
Bilirubin, conjugated	20 mg/dL
Bilirubin, unconjugated	20 mg/dL
Haemoglobin	200 mg/dL
Triglyceride	600 mg/dL

- **Serum-plasma study**
  The 17-a-OH-Progesterone ELISA matrix comparison study was performed to evaluate the difference across tube types (serum separator tubes (SST), lithium heparin plasma, sodium heparin plasma and K2 EDTA plasma) versus the control samples (red top serum, without additive) following CLSI (EP9-A) guidelines. A total of 27 samples (23 native, 4 spiked) to cover the assay range were evaluated. Passing-Bablok regression analysis was performed on the comparative data:

Sample type	Slope (95% CI)	Intercept [ng/mL] (95% CI)	Correlation coefficient (r)
SST	1.02 (0.91-1.13)	-0.03 (-0.21-0.11)	0.99
Lithium Heparin	1.00 (0.87-1.04)	0.00 (-0.07-0.08)	0.99

Sodium Heparin	1.00 (0.94-1.10)	-0.02 (-0.14-0.03)	0.99
EDTA	1.04 (0.90-1.12)	-0.03 (-0.19-0.09)	0.99

TRACEABILITY

The 17-a-OH-Progesterone ELISA has been standardized against internal reference standards (serum matrix) which have been value assigned to a commercially available LCMS/MS method.

EXPECTED VALUES

The following ranges were determined using the 17-a-OH-Progesterone ELISA and are provided for information only. The 95 % reference interval for apparently healthy adults were calculated by a non-parametric method following guidance from CLSI C28-A “Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory”.

		n	Median [ng/mL]	Reference Interval [ng/mL]
Women	luteal phase	123	0.45	<LoQ – 2.19
	follicular phase	122	0.32	<LoQ – 2.10
	post-menopausal	122	<LoQ	<LoQ – 0.95
Men		122	0.75	<LoQ – 1.97
Children		124	<LoQ	<LoQ – 1.48

The above ranges should be considered as guidelines only; it is recommended that each laboratory establish its own expected range based upon its own patient population.

LIMITATIONS

- As in the case of any diagnostic procedure, results must be interpreted in conjunction with the patient’s clinical presentation and other information available to the physician.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays[9]. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed.

WASTE MANAGEMENT

Reagents must be disposed of in accordance with local regulations.

All materials that have come into contact with samples and reagents must be disposed of in accordance with country, state and local regulations.

LITERATURE

1. Honour JW. 17-Hydroxyprogesterone in children, adolescents and adults. Ann Clin Biochem. 2014, Jul;51(Pt 4):424-40.
2. Speiser PW, Arlt W, Auchus RJ, Baskin LS, Conway GS, Merke DP, Meyer-Bahlburg HFL, Miller WL, Murad MH, Oberfield SE, White PC. Congenital Adrenal Hyperplasia Due to Steroid 21-Hydroxylase Deficiency: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2018 Nov 1;103(11):4043-4088.
3. Bornstein SR, Allolio B, Arlt W, Barthel A, Don-Wauchope A, Hammer GD, Husebye ES, Merke DP, Murad MH, Stratakis CA, Torpy DJ. Diagnosis and Treatment of Primary Adrenal Insufficiency: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2016 Feb;101(2):364-89.
4. Sharma R, Seth A. Congenital adrenal hyperplasia: issues in diagnosis and treatment in children. Indian J Pediatr. 2014 Feb;81(2):178-85.
5. Legro RS, Arslanian SA, Ehrmann DA, Hoeger KM, Murad MH, Pasquali R, Welt CK; Endocrine Society. Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2013
6. Dec;98(12):4565-92. 6. Grandone A, Marzuillo P, Luongo C, Toraldo R, Mariani M, Miraglia Del Giudice E, Perrone L. Basal levels of 17-hydroxyprogesterone can distinguish children with isolated precocious pubarche. Pediatr Res. 2018 Oct;84(4):533-536.
7. Rachoń D. Differential diagnosis of hyperandrogenism in women with polycystic ovary syndrome. Exp Clin Endocrinol Diabetes. 2012 Apr;120(4):205-9.
8. Basic QC Practices On-line Course; http://www.Westgard.com.
9. Boscato, LM. and Stuart, MC., ‘Heterophilic antibodies: a problem for all immunoassays’. Clin Chem, 34, 1988, pp 27–33

USED SYMBOLS

Symbol	Description
Cont.	Content
CE	
IVD	
2°C 8°C	