ab108668 – 17 OH Progesterone ELISA Kit

Instructions for Use

A competitive immunoenzymatic assay for the quantitative measurement of 17 OH Progesterone in serum and plasma (citrate).

This product is for research use only and is not intended for diagnostic use.

Version 2 Last Updated 5 February 2019

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1. BACKGROUND

Abcam's 17 OH Progesterone *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of 17 OH Progesterone in Human serum and plasma.

A 96-well plate has been precoated with anti-17 OH Progesterone IgG. Samples and the 17 OH Progesterone-HRP conjugate are added to the wells, where 17 OH Progesterone in the sample competes with the added 17 OH Progesterone-HRP for antibody binding. After incubation, the wells are washed to remove unbound material and TMB substrate is then added which is catalyzed by HRP to produce blue coloration. The reaction is terminated by addition of Stop Solution which stops the color development and produces a color change from blue to yellow. The intensity of signal is inversely proportional to the amount of 17 OH Progesterone in the sample and the intensity is measured at 450 nm.

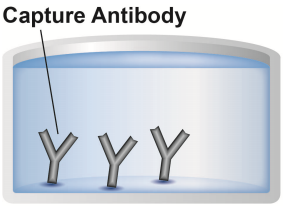
Progesterone 17 alpha OH is a C-21 steroid hormone produced in the adrenal gland and gonads, during the synthesis of glucocorticoids and sex steroids. It is derived from progesterone via 17-hydroxylase, a P450c17 enzyme, or from 17-hydroxypregnenolone via 3β-hydroxysteroid dehydrogenase/Δ5-4 isomerase. Progesterone 17 alpha OH 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. Progesterone 17 alpha OH has no defined physiologic role except as a precursor molecule. Serum Progesterone 17 alpha OH levels are age-dependent, with peak levels observed during fetal life and the immediate postnatal period.

During the first week of life, serum Progesterone 17 alpha OH levels fall ~50-fold compared to umbilical cord blood levels. 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 Progesterone 17-alpha OH levels normally have an ACTH-dependent diurnal variation, with peak levels in the morning and a decline at night. Ovarian

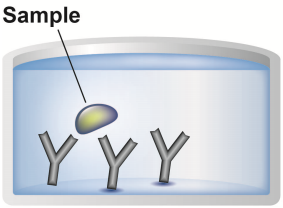
production of Progesterone 17-alpha OH increases during the luteal phase of the menstrual cycle. Progesterone 17-alpha OH is a natural progestin and in pregnancy increases in the third trimester primarily due to fetal adrenal production.

Normal levels are 3 – 90 ng/dL in children and in women, 15 – 80 ng/dL prior to ovulation and 35 – 290 ng/dL during the luteal phase. Measurements of levels of Progesterone 17 alpha OH are useful in the evaluation of patients with suspected congenital adrenal hyperplasia as the typical enzymes that are defective, namely 21- hydroxylase and 11 beta-hydroxilase, lead to a build-up of Progesterone 17 alpha OH. In contrast, the rare patient with 17α-hydroxylase deficiency will have very low or undetectable levels of Progesterone 17 alpha OH. Elevated serum Progesterone 17 alpha OH levels at baseline and/or after ACTH stimulation have also been reported in other forms of adrenal hyperplasia.

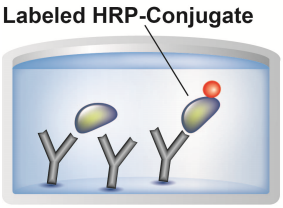
2. ASSAY SUMMARY



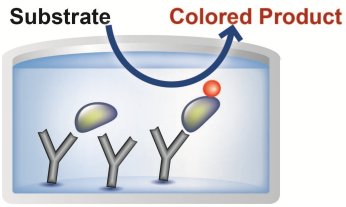
Prepare all reagents, samples, controls and standards as instructed.



Add samples, standards and controls to wells used.



Add prepared labeled HRP-Conjugate to each well. Incubate at 37ºC.



After washing, add TMB substrate solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 2-8°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Anti-17 OH Progesterone IgG Coated Microplate (12 x 8 wells)	96 Wells	2-8°C
Stop Solution	15 mL	2-8°C
17 OH Progesterone-HRP Conjugate	17 mL	2-8°C
TMB Substrate Solution	15 mL	2-8°C
17 OH Progesterone Control	1 mL	2-8°C
17 OH Progesterone Standard 0 – 0 ng/mL	1 mL	2-8°C
17 OH Progesterone Standard 1 – 0.2 ng/mL	1 mL	2-8°C
17 OH Progesterone Standard 2 – 0.6 ng/mL	1 mL	2-8°C
17 OH Progesterone Standard 3 – 2.0 ng/mL	1 mL	2-8°C
17 OH Progesterone Standard 4 – 6.0 ng/mL	1 mL	2-8°C
17 OH Progesterone Standard 5 – 20.0 ng/mL	1 mL	2-8°C
Wash Solution 10X	50 mL	2-8°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm or 620 nm
- Incubator at 37°C
- Multi- and single-channel pipettes to deliver volumes between 10 and 1,000 µL
- Optional: Automatic plate washer for rinsing wells.
- Rotating mixer
- Deionised or (freshly) distilled water.
- Disposable tubes
- Timer

7. LIMITATIONS

- ELISA kit intended for research use only. Not for use in diagnostic procedures
- All components of Human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious
- Use only clean pipette tips, dispensers, and lab ware
- Do not interchange screw caps of reagent vials to avoid cross-contamination
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use

- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate, without splashing, accurately to the bottom of wells

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings
- 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
- 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
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

Equilibrate all reagents, samples and controls to room temperature (18-25°C) prior to use.

9.1 Wash Solution 1X

Prepare 1X Washing Solution by diluting Wash Solution 10X with distilled water. To make 500 mL 1X Wash Solution combine 50 mL Wash Solution 10X with 450 mL distilled water. Mix thoroughly and gently. The Wash Solution 1X is stable for 30 days at 2-8°C. If crystals are present in Wash Solution 10X, mix at room temperature until completely dissolved.

10. SAMPLE COLLECTION AND STORAGE

- The determination of 17 OH Progesterone can be performed in plasma as well as in serum. If the assay is not performed on the same day of collection store the sample at -20°C. If samples are stored frozen, mix thawed samples gently for 5 min. before testing.

  Avoid repeated freezing and thawing

11. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For each assay performed, a minimum of 1 well must be used as a blank, omitting sample and conjugate from well addition
- For statistical reasons, we recommend each standard and sample should be assayed with a minimum of two replicates (duplicates).

12. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described.**
- **If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of washing solution from 300 µL to 350 µL to avoid washing effects.**
- **Assay all standards, controls and samples in duplicate.**

13.1. Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

13.3. Add 25 µL standard, control or sample into their respective wells. Add 200 µL 17 OH Progesterone-HRP Conjugate to each well. Leave a blank well for substrate blank.

13.4. Cover wells with the foil supplied in the kit.

13.5. Incubate for 1 hour at 37°C.

13.6. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL distilled water. Avoid overflows from the reaction wells. During each wash step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent towel.

Automatic washer: In case you use an automatic washer, it is advised to do 6 washing steps.

Note: Washing is critical. Insufficient washing results in poor precision and falsely elevated absorbance values.

13.7. Add 100 µL TMB Substrate Solution into all wells.

13.8. Incubate for exactly 15 minutes at room temperature in the dark.

13.9. Add 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Shake the microplate gently. Any blue color developed during the incubation turns into yellow.

13.10. Measure the absorbance of the sample at 450 nm against a reference wavelength of 620-630nm or against blanks within 5 minutes of addition of the Stop Solution.

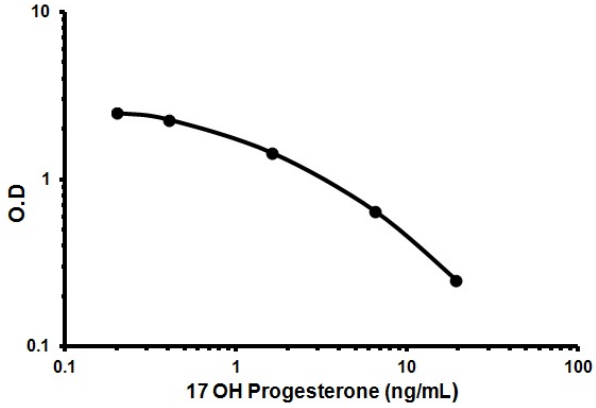
13. CALCULATIONS

Calculate the mean background subtracted absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (e. g.: Four Parameter Logistic).

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

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.



Conc. (ng/mL)	O.D
0	2.91
0.2	2.51
0.4	2.29
1.6	1.45
6.4	0.65
19.2	0.25

15. TYPICAL SAMPLE VALUES

REFERENCE VALUES-

Human serum 17 OH Progesterone reference values:

Women	Follicular phase	0.2 – 1.3 ng/mL
	Luteal 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

SENSITIVITY –

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

PRECISION –

	Intra-Assay	Inter-Assay
n=	3	3
%CV	≤ 8.2	≤ 13.8

RECOVERY –

The recovery test performed on three different samples, enriched with 6.6 – 3.3 – 1.65 – 0.83 – 0.41 ng/mL of 17 OH Progesterone gave an average value (±SD) of 98.66% ± 5.99%. In the dilution test three different samples were diluted 2, 4 and 8 times with standard; the average value (±SD) obtained is 95.31% ± 4.88%.

16. ASSAY SPECIFICITY

The cross reaction of the antibody calculated at 50% is:

17α OH Progesterone	100%
11-Deoxycortisol	0.846%
Progesterone	0.590%
Pregnenolone	0.250%
Testosterone	0.017%
17-beta Estradiol	<0.001%
Aldosterone	<0.001%
Estriol	<0.001%
Estrone 3-sulfate	<0.001%
Spironolactone	<0.001%
Androstenedione	<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%

17. TROUBLESHOOTING

Problem	Cause	Solution
Low signal	Incubation time to short	Try overnight incubation at 4 °C
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed/wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes & ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)

Problem	Cause	Solution
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding stop solution	Read plate immediately after adding stop solution
Low sensitivity	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

18. NOTES

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp