17-OH-Progesterone GENLISA® ELISA

REF : KBD307

Ver 2.5

IVD

ELISA immunoassay for quantitative determination of 17-OH-Progesterone in serum and plasma

IVD	**For Invitro Diagnostic Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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REF KBD307 Σ 96 tests

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Introduction:

The steroid 17-hydroxyprogesterone (17-OHP) is produced by both the adrenal cortex and the gonads. It is synthesised from progesterone and serves primarily as a precursor that is converted into cortisol in the adrenal gland, or into androgenic and estrogenic steroid hormones in the gonads. Although 17-OHP has relatively little progestational activity, it is of significant clinical interest because it is the immediate precursor of 11-deoxycortisol (Compound S). 11-Deoxycortisol is formed by 21-hydroxylation at the C-21 position. Therefore, 21-hydroxylase activity in the adrenal cortex can be assessed by measuring circulating 17-OHP levels.

Intended Use:

The 17-OH-Progesterone GENLISA® ELISA is intended for the quantitative determination of 17-OH-Progesterone in serum and plasma.

Principle:

The 17-OH-Progesterone GENLISA® ELISA method is a quantitative determination based on competitive enzyme-linked immunosorbent assay (ELISA) to determine the level of 17-OH-Progesterone in samples. Standards, Samples and Controls are added to the respective microtiter wells which are pre-coated with polyclonal 17-OHP antibody. 17-OHP conjugated to HRP is added to the microplate to compete with the 17-OHP present in the samples to form a complex. After incubation and a washing step, TMB Substrate is added, which develops a blue color on incubation. The reaction is stopped with a Stop Solution to form a yellow color. The concentration of the 17-OHP molecules in the samples is inversely proportional to the yellow color developed (absorbance) in the wells. The concentration of 17-OH-Progesterone in the sample is calculated through a calibration curve.

Materials Provided:

1. Microtiter Coated Plate (96 wells) - 1 no.
2. Standards (0, 0.2, 0.6, 2, 6, 16 ng/ml) – 1 ml / vial
3. Low Control - 1 ml
4. High Control - 1 ml
5. HRP Conjugate - 22 ml
6. (20X) Wash Buffer – 2 x 25 ml
7. TMB Substrate - 12 ml
8. Stop Solution - 12 ml
9. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Graph paper or software for data analysis.
6. Timer.
7. Absorbent Paper.

Handling/Storage:

1. Store main kit components at recommended storage temperature indicated on the component label.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling:

Serum- Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, centrifuge again.

Plasma- Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, centrifuge again.

Reagent Preparation:

1. Allow all components to reach RT (Room Temperature) prior to use in the assay.
2. (1X) Wash Buffer Dilution: To make (1X) Wash Buffer, add 25ml of (20X) Wash Buffer to 475ml of DI water. This is the working solution.

Test Procedure:

1. All reagents should be allowed to reach room temperature before use.

2. Add **25 ul Standard, Sample** and **Controls** into respective wells in sequence.

3. Add **200 ul** of **HRP Conjugate** to each well except the blank well. Gently mix. Seal the plate using the sealing membrane.

4. Incubate at **37ºC** for 60 minutes.

5. Aspirate and wash plate 6 times with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.

6. Add **100 ul** of **TMB Substrate to all wells**.

7. Incubate at **Room Temperature** for 15 minutes in the dark.

8. Add **100 ul** of **Stop Solution to all wells**, mix well. Read result with an ELISA reader at 450 nm.

Interpretation of Results:

Determine the Mean Absorbance (net of Blank) for each set of duplicate Standards and Samples. Using graph paper, plot the average value (absorbance 450 nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the unknown Concentration. If samples were diluted, multiply by the appropriate dilution factor.

Software which is able to generate a 4-PL (2nd order), or cubic spline is best recommended for automated results.

Note:
It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard.
- If the determination value is higher or lower than normal range, it means there is an abnormal result. The final

result should be diagnosed in correlation with the clinical symptoms and other diagnostic methods.

Validity of the Test:

The test is valid if following conditions are met.

Low Control: 0.45 – 1.08 ng/ml.
High Control: 2.90 – 6.16 ng/ml.

Calculation of results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using Graph Paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the unknown concentration.

If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit or 4-PL is best recommended for automated results.

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".

Expected values:
The following ranges were determined using the 17-OH-Progesterone ELISA and are provided for information only. The 95 % reference interval for apparently healthy adults was 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 (3 – 18 years)	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.

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

Detection Capability:
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.

Sensitivity	Concentration
Limit of Blank (LoB)	0.02 ng/ml
Limit of Detection (LoD)	0.11 ng/ml
Limit of Quantitation (LoQ)	0.26 ng/ml

Precision:
Precision of the 17-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 Conc. (ng/ml)	Within run (Repeatability)	
			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: 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 Conc. (ng/ml)	Within run (Reproducibility)	
			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-OH-Progesterone concentration by 17-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 %.

Analytical Specificity:
The specificity was assessed with the following cross reactants.

Cross-reactant	Concentration tested (unit)	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-OH-Progesterone ELISA when the concentrations are below the stated threshold presented in the following table.

Potentially Interfering Reagent	Threshold Concentration
Bilirubin, conjugated	20 mg/dl
Bilirubin, unconjugated	20 mg/dl
Haemoglobin	200 mg/dl
Triglyceride	600 mg/dl

Serum-plasma study:
The 17-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 to 1,13]	-0.03 [-0,21 to 0,11]	0.99
Lithium Heparin	1.00 [0,87 to 1,04]	0.00 [-0,07 to 0,08]	0.99
Sodium Heparin	1.00 [0,94 to 1,10]	-0.02 [-0,14 to 0,03]	0.99
EDTA	1.04 [0,90 to 1,12]	-0.03 [-0,19 to 0,09]	0.99

Safety Precautions:

- This kit is For In Vitro Diagnostic Use only. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:
- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

LIMITED WARRANTY

Krishgen Pudgala LLP does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by Krishgen Pudgala LLP, or against damages resulting from such non-Krishgen Pudgala LLP made products or components. Krishgen Pudgala LLP passes on to customer the warranty it received (if any) from the maker thereof of such non-Krishgen made products or components. This warranty also does not apply to product to which changes, or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Pudgala LLP.

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Krishgen Pudgala LLP, 2026.

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Krishgen Pudgala LLP

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Regulatory Status:

CE Marked	Europe
FDA registered	USA
CDSCO registered	India

SCHEMATIC ASSAY PROCEDURE

1	All reagents should be allowed to reach room temperature before use.
2	Add **25 ul Sample, Standards, Controls** into respective wells in sequence.
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4	Incubate at **37ºC** for 60 minutes.
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SYMBOLS KEY

MTP	**Microtiter Plate (96 wells)**
Low CNTRL	**Low Control**
High CNTRL	**High Control**
STD	**Standards**
ENZY CONJ	**Enzyme Conjugate**
20x WASH BUF	**(20x) Wash Buffer**
SUB TMB	**TMB Substrate**
SOLN STOP	**Stop Solution**
	**Consult Instructions for Use**
REF	**Catalog Number**
	**Expiration Date**
	**Storage Temperature**