17-OH-Progesterone GENLISA™ ELISA

REF : KBD307

Ver 2.1

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**

REF KBD307 Σ 96 tests

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Introduction:

The steroid 17 Hydroxyprogesterone (17-OH P) is produced by both the adrenal cortex and gonads. It is synthesised from progesterone and serves primarily as a precursor compound that is converted into cortisol in the adrenal gland, or into androgenic and estrogenic steroid hormones in the gonads. Even though 17-a-OHP has relatively little progestational activity, it is of intense clinical interest because it is the immediate precursor to 11-desoxycortisol (Cpd-S). CpS is formed by hydroxylation of the carbon atom C 21. Enzyme activity of 21-hydroxylase in the adrenal cortex may thus be monitored by analyzing the level of 17-OHP in the blood.

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 is pre-coated with polyclonal 17-α-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-α-OHP molecules in the samples is inversely proportional to the yellow color developed (absorbance) in the wells. The concentration of 17OH Progesterone in the sample is calculated through a calibration curve.

Materials Provided:

1. Microtiter Coated Plate (96 wells) - 1 no
2. Standards (1 ml/vial) - 0, 0.2, 0.6, 2, 6, 20 ng/ml
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** into every well except blank well.
7. Incubate at **Room Temperature** for 15 minutes in the dark.
8. Add **100 ul** of **Stop Solution** except the blank well, 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: 0.36 – 0.66 ng/ml
High Control: 3.05 – 5.66 ng/ml

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.

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