Human Pregnenolone ELISA Kit

Enzyme Immunoassay for the quantification of Human Pregnenolone in serum.

Catalog number: ARG81044

Package: 96 wells

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INTRODUCTION

Pregnenolone (P5), or pregn-5-en-3β-ol-20-one, is an endogenous steroid and precursor/metabolic intermediate in the biosynthesis of most of the steroid hormones, including the progestogens, androgens, estrogens, glucocorticoids, and mineralocorticoids. In addition, pregnenolone is biologically active in its own right, acting as a neurosteroid.

In addition to its role as a natural hormone, pregnenolone has been used as a medication and supplement; for information on pregnenolone as a medication or supplement, see the pregnenolone (medication) article.

Pregnenolone and its 3β-sulfate, pregnenolone sulfate, like DHEA, DHEA sulfate, and progesterone, belong to the group of neurosteroids that are found in high concentrations in certain areas of the brain, and are synthesized there. Neurosteroids affect synaptic functioning, are neuroprotective, and enhance myelinization. Pregnenolone and its sulfate ester may improve cognitive and memory function. In addition, they may have protective effects against schizophrenia. [Provided by Wikipedia: Pregnenolone]

PRINCIPLE OF THE ASSAY

This assay employs the competitive quantitative enzyme immunoassay technique. A highly specific antibody for Pregnenolone has been pre-coated onto a microplate. Pregnenolone containing samples or standards and a Pregnenolone-HRP conjugate are given into the wells of the microtiter plate. Enzyme labeled and free Pregnenolone compete for the antibody binding sites. After incubation at room temperature, the wells are washed with diluted Wash Buffer to remove unbound material. Then TMB substrate is added to the wells and color develops in inversely proportion to the amount of Pregnenolone present in the samples. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450 nm. The concentration of Pregnenolone in the samples is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all other components at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated microplate	8 X 12 strips	4°C
Standard A (0 ng/mL)	2.0 mL (ready to use)	4°C
Standards B to F (0.1, 0.4, 1.6, 6.4, 25.6 ng/mL)	0.5 mL each (ready to use)	4°C
Control 1 & 2	0.5 mL each (ready to use)	4°C
Diluent Buffer	15 mL (ready to use)	4°C
50X Pregnenolone-HRP Conjugate	300 µL per vial	4°C
10X Wash Buffer	50 mL	4°C
TMB substrate	16 mL (ready to use)	4°C (protect from light)
STOP solution	6 mL (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- Mixer or Ultra-Turrax
- Microplate shaker
- Pipettes and pipette tips
- Microtiter plate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature (20-25°C).
- Remove the number of strips required and return unused strips to the pack and reseal.
- Avoid air bubbles in the wells as this could result in lower binding efficiency and higher CV% of duplicate reading.
- Briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.

- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Avoid disturbing the white buffy layer when collection serum/plasma sample.
3. Samples containing sodium azide should not be used in the assay.
4. Specimens should be capped and may be stored for up to 24 hours at 2-8°C prior to assaying. Specimens stored for a longer time (up to 3 months) should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.
5. If a sample reads more than 25.6 ng/mL then dilute it with Standard A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

REAGENT PREPARATION

- **1X Wash Buffer:** Dilute 10X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 50 mL of 10X Wash Buffer into 450 mL of distilled water to a final volume of 500 mL) The 1X Wash Buffer is stable for up to 4 weeks at 2-8°C.
- **1X Pregnenolone-HRP Conjugate:** Dilute 1:50 in assay buffer before use (E.g. 40 µL of HRP in 2 mL of Diluent Buffer). If the whole plate is to be used dilute 240 µL of HRP in 12 mL of Diluent Buffer. Discard any that is left over.

ASSAY PROCEDURE

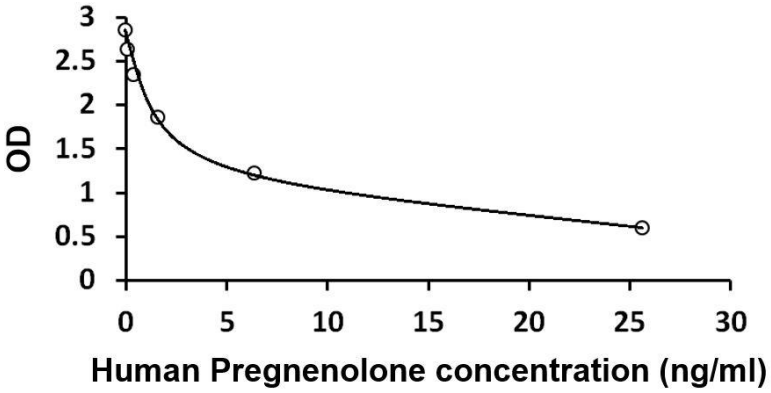
All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and samples should be assayed in duplicates.

1. Add **50 µL** of **Standards, Controls and samples** into the appropriate wells of the Antibody Coated Microplate.
2. Add **100 µL** of **1X Pregnenolone-HRP Conjugate** into all wells.
3. Incubate at **RT** for **60 minutes** on a microplate shaker.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add **150 µL** of **TMB Substrate** to each well, including the blank wells. Incubate in the dark for **10-15 minutes** at **RT** on a microplate shaker.

6. Immediately Add 50 µL of Stop Solution to each well, including the blank wells. The color of the solution should change from blue to yellow.
7. Read the OD with a microplate reader at 450 nm immediately. (optional: read at 620 nm as reference wavelength) It is recommended reading the absorbance within 20 minutes after adding the stop solution.

EXAMPLE OF TYPICAL STANDARD VALUES

The following figures demonstrate typical results with the Human Pregnenolone ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of Controls, standards and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
4. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

QUALITY ASSURANCE

Sensitivity

The sensitivity of the Human Pregnenolone ELISA kit is 0.05 ng/ml.

Specificity

Substance	Cross Reactivity (%)
Pregnenolone	100
Progesterone	6.0
Dehydroisoandrosterone	5.2
5α-Androstandiol	4.7
Epiandrosterone	1.0
Pregnenolone Sulfate	0.4
Androstandione	0.3
5α-Androsterone	0.3
DHEAS	0.2
Etiocholanolone	0.1

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 7.8-10.6% and CV value of inter-assay precision was 9.6-14.5%.

Recovery

107.2-120.2%