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17-Hydroxyprogesterone ELISA Kit

08/17

(Catalog # E4345-100, 100 assays, Store at 4°C)

I. Introduction:

The adrenal glands, ovaries, testes, and placenta produce 17-hydroxyprogesterone. It is hydroxylated at the 11 and 21 position to produce cortisol. Deficiency of either 11- or 21-hydroxylase results in decreased cortisol synthesis, and feedback inhibition of adrenocorticotropic hormone (ACTH) secretion is lost. Consequent increased pituitary release of ACTH will increase production of 17-HOP. But, if 17-alpha-hydroxylase (which allows formation of 17-HOP from progesterone) or 3ß-hydroxysteroid dehydrogenase type 2 (which allows formation of 17-hydroxyprogesterone formation from 17-hydroxypregnenolone) are deficient, 17-HOP levels are low with possible increase in progesterone or pregnenolone respectively. BioVision's 17-Hydroxyprogesterone ELISA kit is a competitive ELISA assay for the quantitative measurement of 17-Hydroxyprogesterone in extracted serum and plasma, or in urine, extracted dried fecal samples, and tissue culture media samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of 17-Hydroxyprogesterone.
Detection Range: 6000 – 24.69 pg/ml
Sensitivity: < 20.3 pg/ml
Detection Limit: 15.4 pg/ml

III. Specificity:

Universal

IV. Sample Type:

Urine, tissue culture samples, extracted serum and plasma, or extracted dried fecal samples

V. Kit Contents:

Components	E4345-100	Part No.
Micro ELISA Plate	8 X 12 strips	E4345-100-1
Standard	70 µl	E4345-100-2
17-Hydroxyprogesterone Antibody	3 ml	E4345-100-3
17-Hydroxyprogesterone Conjugate	3 ml	E4345-100-4
Assay Buffer Concentrate (5X)	28 ml	E4345-100-5
Wash Buffer Concentrate (20X)	30 ml	E4345-100-6
TMB Substrate	11 ml	E4345-100-7
Stop Solution	5 ml	E4345-100-8
Plate Sealer	1	E4345-100-9

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Ethyl acetate or ethanol for serum, plasma or fecal extracts
- Speedvac for evaporation of ethanol or ethyl acetates
- Precision pipettes with disposable tips

VII. Storage and Handling:

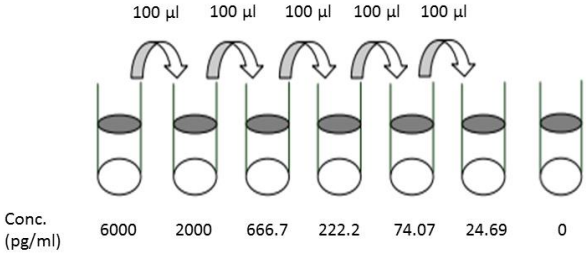
The entire kit may be stored at 4°C for up to 6 months. Avoid freeze-thaw cycles.

VIII. Reagent Preparation:

Note: Prepare reagents within 30 minutes before the experiment.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Assay Buffer:** Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.
2. **Wash Buffer**: Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable for 3 months at room temperature.
3. **Standard Preparation:**

- Add 20 µl of the 17-Hydroxyprogesterone stock solution to 380 µl of Assay Buffer (tube #1) and vortex completely.
- Prepare 4 vials of standards (tube #2-6) by adding 0.1 ml of the above stock solution in 0.2 ml of Assay Buffer. Perform 3-fold serial dilutions of the top standards to make the standard curve within the range of this assay.
- Suggested standard points are: 6000, 2000, 666.7, 222.2, 74, and 24.69 pg/ml.



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- Use all Standards within 2 hours of preparation.

4. Sample Preparation:

Note: Use all Samples within 2 Hours of preparation, or stored at ≤ -20°C until assaying. Avoid multiple freeze-thaw cycles.

- **Extracted serum and plasma:** Add diethyl ether or ethyl acetate to serum or plasma samples at a 5:1 (v/v) solvent sample ratio. Mix solutions by vortexing for 2 minutes. Allow layers to separate for 5 minutes. Freeze samples in a dry ice/ethanol bath and pipet off the solvent solution from the top of the sample into a clean tube. Repeat steps 1-3 for maximum extraction efficiency, combining the solvent solutions. Dry pooled solvent extracts down in a speedvac for 2-3 hrs. If samples need to be stored they should be kept at -20°C. Redissolve samples at room temperature in diluted Assay Buffer. A minimum of 125 µl of Assay Buffer is recommended for reconstitution to allow for duplicate sample measurement.
- **Urine**: Urine samples should be diluted at least 1:2 in diluted Assay Buffer.
- **Tissue Culture Media:** For measuring 17-HOP in tissue culture media (TCM), samples should be read off a standard curve generated in TCM. Samples may need to be diluted further in TCM.
- End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit.

IX. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VIII.
2. Pipet 50 µl of samples or standards into wells in the plate. Pipet 75 µl of Assay Buffer into the non-specific binding (NSB) wells.
3. Add 25 µl of the 17-Hydroxyprogesterone Conjugate to each well. Add 25 µl of the 17-Hydroxyprogesterone Antibody to each well, except the NSB wells.
4. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and shake at room temperature for 1 hour.
5. Aspirate the plate and wash each well 4 times with 300 µl wash buffer. Tap the plate dry on clean absorbent towels.
6. Add 100 µl of the TMB Substrate to each well. Incubate the plate at room temperature for 30 minutes.
7. Add 50 µl of the Stop Solution to each well.
8. Read the optical density at 450 nm within 15 minutes.

X. CALCULATION:

Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean OD's for the non-specific binding well (NSB). The sample concentrations obtained, calculated from the $\%B/B_0$ curve, and should be multiplied by the dilution factor to obtain neat sample values.

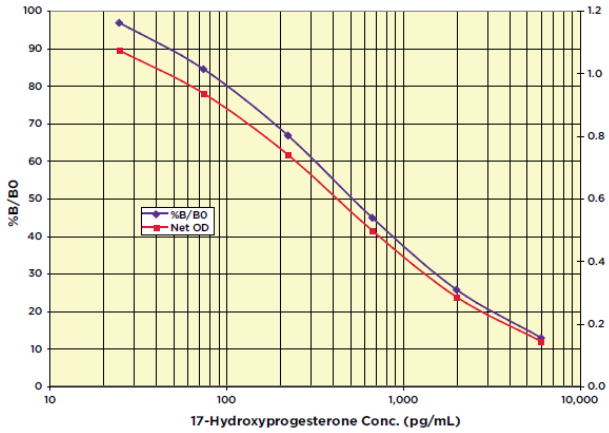

Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

XI. VALIDATION DATA:

Linearity: and Recovery Rate:

High Urine	Low Urine	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
80%	20%	4,355	4,824	110.8
60%	40%	3,330	3,789	113.8
40%	60%	2,304	2,623	113.8
20%	80%	1,278	1,543	120.8
			Mean Recovery	**114.8%**

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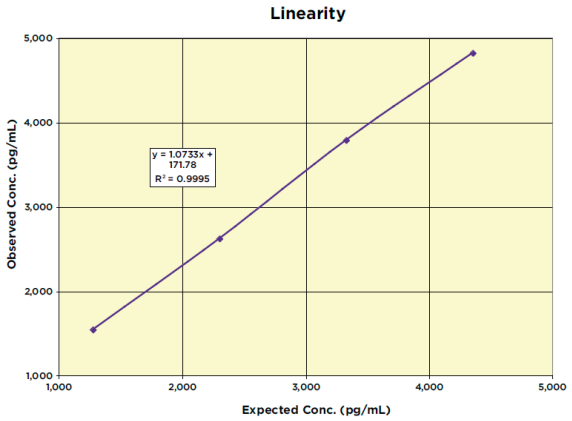

Intra Assay:

Sample	17-Hydroxyprogesterone Conc. (pg/mL)	%CV
1	1,265.7	5.4
2	450.0	6.5
3	168.5	7.9

Inter Assay Precision:

Sample	17-Hydroxyprogesterone Conc. (pg/mL)	%CV
1	1,204.8	7.0
2	444.1	6.5
3	162.9	10.6

Cross Reactivity:

Steroid	Cross Reactivity (%)
17-Hydroxyprogesterone	100%
17α-Hydroxypregnanolone	17.4%
Progesterone	0.29
11α-Hydroxyprogesterone	0.08
5α-dihydroprogesterone	0.04
20α-Hydroxyprogesterone	< 0.01
Androstendione	< 0.01
Cholesterol	< 0.01
Corticosterone	< 0.01
Cortisol	< 0.01
Pregnenolone	< 0.01

XII. RELATED PRODUCTS:

- Progesterone receptor (PGR) (Human) ELISA Kit (Cat. No. K4270)
- Progesterone (human) ELISA Kit (Cat. No. K7414)
- Progesterone ELISA Kit (Cat. No. K7416)
- Progesterone (Cat. No. K2913)
- Cortisol (human/mouse/rat) ELISA Kit (Cat. No. 7430)
- Corticosterone (CORT) ELISA Kit (Cat. No. K4222)

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