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Epiandrosterone ELISA Kit

07/20

(Catalog # E4852-100, 96 assays, Store at 4°C)

I. Introduction:

Epiandrosterone, or 3ß-androsterone, also known as 3ß-hydroxy-5a-androstan-17-one or 5α-androstan-3ß-ol17-one, is a steroid hormone with weak androgenic activity. It is a natural metabolite of dehydroepiandrosterone (DHEA) via the 5α-reductase enzyme. BioVision's Epiandrosterone ELISA kit is a competitive ELISA assay for the quantitative measurement of Epiandrosterone in urine, saliva, and fecal samples, or in extracted serum and plasma. Standards or diluted samples are pipetted into a clear microtiter plate coated with an antibody to capture rabbit antibodies. An Epiandrosterone-peroxidase conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of a polyclonal antibody to Epiandrosterone to each well. After incubation the plate is washed and substrate is added. The substrate reacts with the bound Epiandrosterone -peroxidase conjugate. After a short incubation, the reaction is stopped and the intensity of the generated color is detected in a microtiter plate reader capable of measuring 450 nm wavelength.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Epiandrosterone.
Detection Range: 0.098 - 100 ng/mL
Sensitivity: 0.120 ng/mL
Detection Limit: 0.107 ng/mL

III. Specificity:

Universal

IV. Sample Type:

Saliva, Urine, Extracted Serum or Plasma, Dried Fecal Extracts and Tissue Culture Media

V. Kit Contents:

Components	E4852-100	Part No.
Micro ELISA Plate	8 X 12 strips	E4852-100-1
Standard	125 µl	E4852-100-2
Epiandrosterone Antibody	3 ml	E4852-100-3
Epiandrosterone Conjugate	3 ml	E4852-100-4
Assay Buffer (5X)	28 ml	E4852-100-5
Wash Buffer (20X)	30 ml	E4852-100-6
TMB Substrate	11 ml	E4852-100-7
Stop Solution	5 ml	E4852-100-8
Plate Sealer	1	E4852-100-9

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Diethyl ether or ethyl acetate for extraction of serum or plasma samples
- Ethanol or methanol will be needed for extraction of fecal samples.
- Precision pipettes with disposable tips

VII. Storage and Handling:

The entire kit may be stored at 4°C for up to 6 months.

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 40 µl of the Epiandrosterone stock solution to 360 µl of Assay Buffer (tube #1) and vortex completely.
- Prepare 7 vials of standards (tube #2-6) by adding 300 µl of Assay Buffer. Take 100 µl of the Epiandrosterone solution from above vial #1 and add to vial #2. Vortex thoroughly. Perform serial dilutions for vial #3 to #8 to make the standard curve within the range of this assay.
- Suggested standard points are: 100, 25, 6.25, 1.563, 0.391 and 0.098 ng/mL.
- Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Assay Buffer (µL)	**360**	300	300	300	300	300
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5
Vol of Addition (µL)	**40**	100	100	100	100	100
Final Conc (ng/mL)	100	25	6.25	1.563	0.391	0.098

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 to serum or plasma samples at a 5:1 (v/v) ether:sample ratio. Mix solutions by vortexing for 2 minutes. Allow ether layer to separate for 5 minutes. Freeze samples in a dry ice/ethanol bath and pipet off the ether solution from the top of the sample into a clean tube. Repeat steps 1-3 for maximum extraction efficiency, combining top layer of ether solutions. Dry pooled ether samples 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 the Assay Buffer. A minimum of 125 µL of the Assay Buffer is recommended for reconstitution to allow for duplicate sample measurement.
- **Urine**: Urine samples should be diluted at least 1:5 with the provided Assay Buffer.
- **Saliva**: Saliva samples should be diluted at least 1:4 in diluted Assay Buffer
- **Tissue Culture Media:** For measuring Epiandrosterone 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 depending on Epiandrosterone levels.
- **Dried Fecal Samples:** Ensure that the sample is completely dry and powder the sample to improve extraction recovery. Remove any large particles, such as grass if possible. We suggest checking the efficiency of extraction by preparing a steroid solution of known concentration in the kit Assay Buffer (AB). Please refer to Extraction Efficiency section below for details. Extract samples and Extraction Efficiency Controls with ethanol or ethyl acetate as follows:
  1. Weigh out ≥ 0.2 gm of dried fecal solid into a tube. Samples can be dried by passive drying, gentle heating (≤ 60°C), or freeze-drying (lyophilization).
  2. Add 1 mL of ethanol (or ethyl acetate) for every 0.1 gm of solid (0.1 gm fecal solid/mL) and seal.
  3. Shake vigorously for at least 30 minutes.
  4. Centrifuge samples at 5,000 rpm for 15 minutes at 4°C. Reserve supernatant in a clean tube. This material can be stored at ≤ -20°C for at least a month if properly sealed**.**
  5. These are general guidelines, please adjust volumes as required. Add 100 µL of extracted sample supernatant from step 4 into a minimum volume of 400 µL assay buffer (Total Reconstitution Vol. = 100µL+400µL). Vortex well and allow to rest 5 minutes at room temperature. Vortex and let rest for 5 minutes twice more to ensure complete steroid solubility.

  Note: The ethanol concentration in the final Assay Buffer dilution added to the well should be ≤ 5%

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 Epiandrosterone Conjugate to each well. Add 25 µl of the Epiandrosterone 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 2 hours. If the plate is not shaken signals bound will be approximately 20% lower.
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 without shaking.
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.

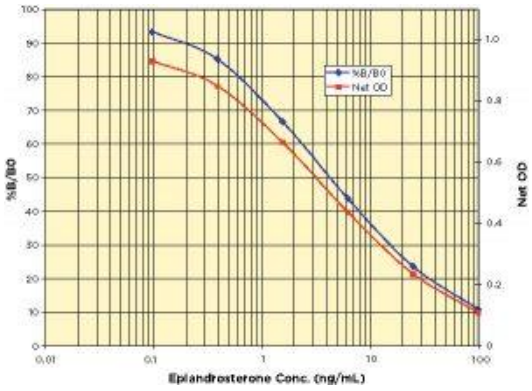

Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

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XI. VALIDATION DATA:

Linearity: Linearity was determined by taking two urine samples diluted with Assay Buffer, one with a low Epiandrosterone level of 0.675 ng/mL and one with a higher level of 24.9 ng/mL, and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used. Linearity in fecal extracts was determined in a similar manner.

High Urine	Low Urine	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
80%	20%	20.06	20.62	102.8
60%	40%	15.21	13.89	91.3
40%	60%	10.37	9.92	95.7
20%	80%	5.52	5.92	107.2
			Mean Recovery	**99.3%**

High Fecal Sample	Low Fecal Sample	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
80%	20%	43.40	44.14	101.7
60%	40%	35.45	34.93	98.5
40%	60%	27.50	26.42	96.0
20%	80%	19.55	18.15	92.8
			Mean Recovery	**97.3%**

Intra Assay: Five human urine samples were diluted with Assay Buffer and run in replicates of ≥ 19 in an assay. The mean and precision of the calculated epiandrosterone concentrations were:

Sample	Epiandrosterone Conc. (ng/mL)	%CV
1	13.84	6.4
2	7.81	9.5
3	5.45	7.9
4	2.45	9.6
5	2.08	8.1

Inter Assay Precision: Five human urine samples were diluted with Assay Buffer and run in duplicates in ≥ 21 assays run over multiple days by four operators. The mean and precision of the calculated epiandrosterone concentrations were:

Sample	Epiandrosterone Conc. (ng/mL)	%CV
1	13.84	10.1
2	8.17	16.2
3	5.34	14.8
4	2.25	18.8
5	1.97	19.8

Cross Reactivity: The following cross reactants were tested in the assay and calculated at the 50% binding point.

Steroid	Cross Reactivity (%)
Androstenedione	161.10
Epiandrosterone glucuronide	112.5
Androsterone	36.5
Dehydroeipandrosterone (DHEA)	33.8
Epiandrosterone sulphate	32.4
Androsterone sulphate	11.8
DHEA sulphate	11.8
Andrenosterone	4.54
19-Nortestosterone	2.3
Progesterone	2.1
DHT	1.9
Testosterone	1.8
Estrone	0.75
17b-Estradiol	0.14
Cortisol	0.12
Corticosterone	0.1

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XII. RELATED PRODUCTS:

- Progesterone (human) ELISA Kit (K7414)
- Progesterone receptor (PGR) (Human) ELISA Kit (K4270)
- Estradiol (rat) ELISA Kit (K3831)
- Estrogen (Human) ELISA Kit (K4264)
- Androstenedione ELISA Kit (E4615)