Epiandrosterone Competitive ELISA Kit

Catalog Number EIAANDO (96 tests)

Pub. No. MAN0018779 **Rev** A.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Epiandrosterone ELISA Kit is designed to detect and quantify the level of epiandrosterone in urine and saliva samples, extracted serum and plasma samples, dried fecal extracts, and tissue culture media.

The assay recognizes epiandrosterone independent of species.

Contents and storage

Kit and components are shipped at -20°C. Upon receipt, store the kit at -20°C. Once open, store the kit at 4°C and use within 2 weeks.

Components	Quantity
Coated Clear 96-Well Plate, clear plastic microtiter plate coated with goat anti-rabbit IgG	1 plate
Epiandrosterone Standard; 1,000 ng/mL	125 µL
Epiandrosterone Antibody	3 mL
Epiandrosterone Conjugate	3 mL
Assay Buffer Concentrate (5X)	28 mL
Wash Buffer Concentrate (20X)	30 mL
TMB (Tetramethylbenzidine) Substrate	11 mL
Stop Solution; contains 1 M HCl, **CAUSTIC**	5 mL
Plate Sealer	1

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm (preferably with correction between 570 nm and 590 nm).
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution
- Diethyl ether or ethyl acetate for extraction of serum or plasma samples.
- Ethanol or methanol for extraction of fecal samples.

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.
- Solutions containing sodium azide will inhibit the activity of the peroxidase conjugate. Ensure that there is no contamination of labware or the plate washer with azide containing solutions.

Prepare 1X Wash Buffer

1. Dilute Wash Buffer Concentrate 1:20 by adding 1 part of the Concentrate to 19 parts of deionized or distilled water. Label as 1X Wash Buffer.
2. 1X Wash Buffer is stable at room temperature for 3 months.

Prepare 1X Assay Buffer

1. Dilute Assay Buffer Concentrate 1:5 by adding 1 part of the Concentrate to 4 parts of deionized or distilled water. Label as 1X Assay Buffer.
2. Store the Concentrate and 1X Assay Buffer in the refrigerator. 1X Assay Buffer is stable at 4°C for 3 months.

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera.
- If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Prepare samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

Use all samples within **2 hours** of dilution, or store at –20°C or lower until ready to perform assay.

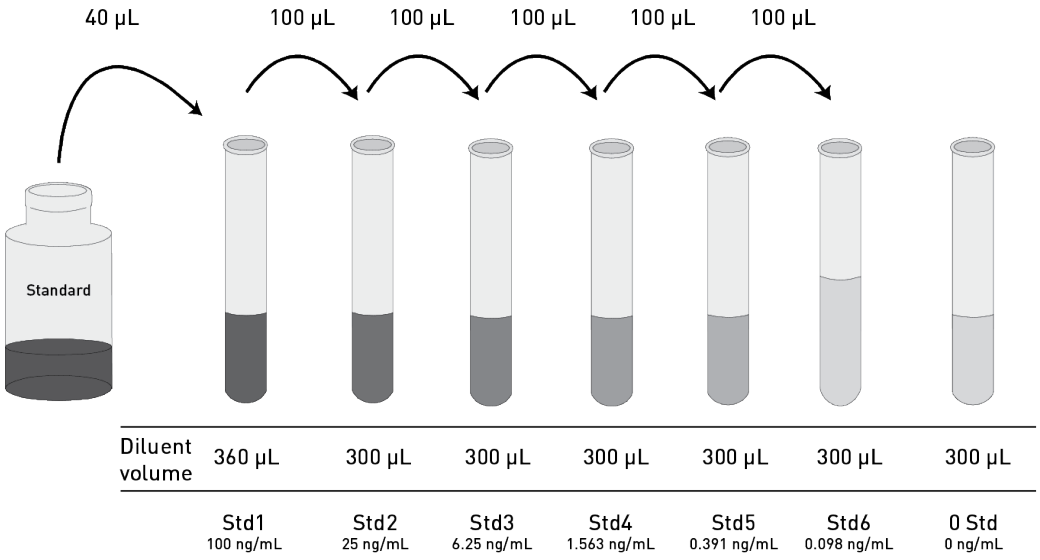
Sample type	Procedure
Serum and plasma	1. Add diethyl ether or ethyl acetate to samples at a 5:1 (v/v) solvent:sample ratio. 2. Mix solutions by vortexing for 2 minutes. Allow solvent layer to separate for 5 minutes. 3. Freeze samples in a dry ice/ethanol bath and pour solvent solution from the top of the sample into a clean tube. Repeat steps 1-3 for maximum extraction efficiency, combining top layer of ether solutions. 4. Dry pooled solvent samples down in a Speedvac for 2-3 hrs. If samples need to be stored they should be kept desiccated at -20°C. 5. Redissolve samples at room temperature in 1X Assay Buffer. Use a minimum of 125 µL of Assay Buffer.
Urine	Dilute samples ≥1:5 with 1X Assay Buffer. **Note**: A Urinary Creatinine Detection Kit (Cat. No. EIACUN) is available for measuring urine creatinine for normalization of epiandrosterone in a random urine specimen.
Dried feces	See detailed extraction protocol on the product page at **thermofisher.com** **Note**: The ethanol concentration in the final diluted Assay Buffer dilution added to the well should be ≤5%.
Saliva	Dilute samples ≥1:4 with 1X Assay Buffer.
Tissue culture media	Dilute samples with tissue culture media (TCM). Concentrations of tissue culture media samples must be determined from a standard curve generated with TCM.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

The Epiandrosterone Standard contains an organic solvent. Pipette the standard up and down several times to wet the pipet tip before transfer to ensure that volumes are accurate.

1. Add 40 µL Epiandrosterone Standard to one tube containing 360 µL 1X Assay Buffer and label as 100 ng/mL Epiandrosterone.
2. Add 300 µL 1X Assay Buffer to each of 6 tubes labeled as follows: 25, 6.25, 1.563, 0.391, 0.098, and 0 ng/mL Epiandrosterone.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
4. **Use the standards within 2 hours of preparation**.

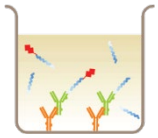


Perform ELISA (Total assay time: 2.5 hours)

IMPORTANT! Perform a standard curve with each assay.

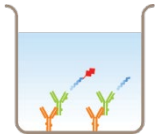
Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.

Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store desiccated at 2°C to 8°C for future use. The silica pack in the bag keeps the plate dry, and turns from blue to pink if the bag is not properly sealed.



Bind antigen

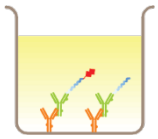
a. Add 50 µL of standards or samples (see "Prepare samples" on page 2) to the appropriate wells.
b. Add 75 µL of 1X Assay Buffer to wells for detecting non-specific binding (NSB).
c. Add 50 µL of 1X Assay Buffer to wells for detecting maximum binding (B0 or zero standard)
d. Add 25 µL of Epiandrosterone Conjugate to each well.
e. Add 25 µL of Epiandrosterone Antibody to each well except NSB wells.
f. Tap the side of the plate to mix. Cover the plate with plate sealer.
g. Shake at room temperature for 2 hours. If the plate is not shaken signals bound will be approximately 20% lower.
h. Thoroughly aspirate the solution and wash wells 4 times with 300 µL of 1X Wash Buffer.



Add chromogen

a. Add 100 µL TMB Substrate to each well. The substrate solution will begin to turn blue.
b. Incubate for 30 minutes at room temperature without shaking.

Note: TMB should not touch aluminum foil or other metals.



Add stop solution

Add 50 µL Stop Solution to each well. Tap side of the plate gently to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 10 minutes after adding the Stop Solution.
2. Average the duplicate Optical Density (OD) values for each standard and sample. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Calculate the concentrations for unknown samples and controls from the %B/B0 curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals lower than that of the highest standard in 1X Assay Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0–100 ng/mL Epiandrosterone.

Epiandrosterone Standard (ng/mL)	Net Optical Density (450 nm)*	%B/B0
100	0.108	10.8
25	0.235	23.5
6.25	0.437	43.8
1.563	0.665	66.6
0.391	0.849	85.1
0.098	0.930	93.2
0	0.998	100

Note: The NSB gave a Mean OD value of 0.081.

Intra-assay precision

Samples were assayed in replicates of ≥19 to determine precision within an assay.

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

CV = Coefficient of Variation

Inter-assay precision

Samples were assayed in duplicates in ≥21 assay runs by four operators to determine precision between assays.

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

CV = Coefficient of Variation

Performance characteristics, continued

Expected values

Multiple human serum and plasma samples were tested in the assay. Adjusted neat concentrations of epiandrosterone for the extracted samples ranged from 33.5 to 55.6 ng/mL with an average 41 ng/mL.

Human urine samples were tested in the assay. Adjusted neat concentrations of epiandrosterone for the urine samples ranged from 121.5 to 1,131 ng/mL with an average of 511.1 ng/mL.

Extracted fecal samples from a male fennec fox read between 25.1 and 1,027 ng/mL.

Sensitivity

The minimum detectable dose of Epiandrosterone is 0.120 ng/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Linearity

Linearity was determined for fecal extracts and diluted urine by taking two samples, one with a low level and one with a higher level of epiandrosterone, and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

Fecal Extract

High Sample %	Low 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%**

Urine

High Sample %	Low Sample %	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%**

Specificity

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
Dehydroepiandrosterone (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

Limited product warranty

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Manufacturer: Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA

The information in this guide is subject to change without notice.