Estriol Competitive ELISA Kit

Catalog Number EIAEST (96 tests)

Pub. No. MAN0018780 **Rev** B.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 Estriol ELISA Kit is a solid-phase competitive Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of estriol in urine, saliva, reconstituted buffer samples, and tissue culture media. The assay can also be used with extracted serum, plasma, and dried fecal samples. The assay recognizes estriol 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. **Store the Estriol Conjugate at –20°C after opening.**

Components	Quantity
Coated Clear 96-well plate; strip-well plate coated with goat anti-rabbit IgG	1 plate
Estriol Standard; 120,000 pg/mL	125 µL
Estriol Antibody	3 mL
Estriol 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, CAUSITC	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 serum or plasma samples)
- Ethanol or methanol (for 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.
- Samples should be considered as potentially infectious and all items which contact the samples as potentially contaminated.

Prepare 1X Wash Buffer

1. Dilute Wash Buffer Concentrate 1:20 by adding 1 part Concentrate to 19 parts of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the Concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 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.
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.
- This kit utilizes a peroxidase-based readout system. Buffers containing sodium azide will inhibit color production from the enzyme. Make sure all buffers used for samples are azide free.
- 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	See detailed extraction protocol on the product page at **thermofisher.com**
Urine	Dilute samples ≥1:8 with 1X Assay Buffer. **Note**: A Urinary Creatinine Detection Kit (Cat. no. EIACUN) is available for comparison to creatinine as a urine volume marker.
Saliva	Dilute samples ≥1:4 with 1X Assay Buffer. See sample handling instructions on the product page at **thermofisher.com**.
Dried feces	See detailed extraction protocol on the product page at **thermofisher.com** **Note**: The ethanol concentration in the final Assay Buffer dilution added to the well should be ≤5%.
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.

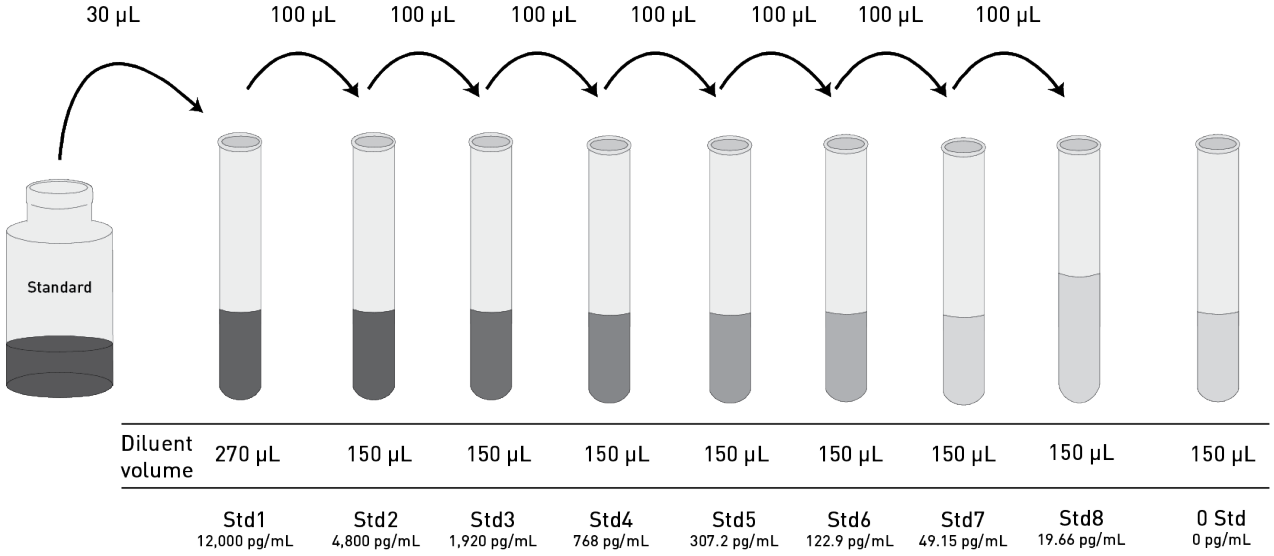
Dilute standards

Note: Use glass or plastic tubes for diluting standards.

The Estriol 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 30 µL Estriol Standard to one tube containing 270 µL 1X Assay Buffer and mix by vortexing. Label as 12,000 pg/mL estriol.
2. Add 150 µL 1X Assay Buffer to each of 8 tubes labeled as follows: 4,800, 1,920, 768, 307.2, 122.9, 49.15, 19.66, and 0 pg/mL estriol.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix by vortexing between steps.

Use the standards within 2 hours of preparation. Discard any remaining reconstituted standard.

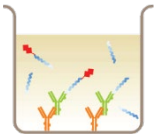


Perform ELISA (Total assay time: 2.5 hours)

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.

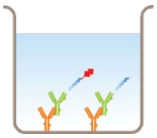


Bind antigen

a. Add 50 µL standards or samples (see "Prepare samples" on page 2) to the appropriate wells.
b. Add 75 µL 1X Assay Buffer to wells for detecting non-specific binding (NSB).
c. Add 50 µL 1X Assay Buffer to wells for detecting maximum binding (B0 or zero standard).
d. Add 25 µL Estriol Conjugate to each well.
e. Add 25 µL Estriol Antibody to each well except NSB wells.
f. Tap the side of the plate to mix, then cover the plate with a plate sealer.
g. Shake at room temperature for 2 hours. If the plate is not shaken signals bound will be ~30% lower.

   OR

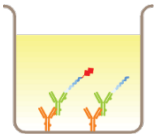
   Shake the plate in a plate shaker at room temperature for 15 minutes to ensure adequate mixing of the reagents. Incubate at 4°C for 16–18 hours without shaking (this option provides the same range for the standard curve, but is about 2-fold more sensitive).
h. Thoroughly aspirate the solution and wash wells 4 times with 300 µL of 1X Wash Buffer.



Add chromogen

a. Add 100 µL TMB Substrate (room temperature) to each well. The substrate solution will begin to turn blue.
b. Incubate for 30 minutes at room temperature.

   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 greater than that of the highest standard in 1X Assay Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (examples)

These data were obtained for the standards over a range of 0–12,000 pg/mL estriol. **Note**: 100 pg/mL of estriol is equivalent to 346.8 pM.

Estriol Standard (pg/mL)	Net OD (450 nm)	%B/B0
12,000	0.267	17.3
4,800	0.324	21.0
1,920	0.423	27.4
786	0.605	39.2
307.2	0.785	50.8
122.9	0.995	64.4
49.15	1.167	75.6
19.66	1.340	86.8
0	1.544	100

Note: The NSB gave a Mean OD value of 0.084.

Intra-assay precision

Five human samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Mean (pg/mL)	1,037	186.2	72.72	634.8	126.6
%CV	8.3	11.3	13.1	8.4	8.4

CV = Coefficient of Variation

Inter-assay precision

Five human samples were assayed in duplicate in 20 assay runs by multiple operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Mean (pg/mL)	1,015	196.1	81.84	631.8	123.2
%CV	10.1	11.6	17.5	10.5	11.0

CV = Coefficient of Variation

Performance characteristics, continued

Expected values

Sample	Range
Urine, neat (male/non-pregnant female)	1.7–66.2 ng/mL
Urine, neat (pregnant female) n=3	1,097– 2,076 ng/mL
Extracted fecal sample	Undetectable to 7,233 pg/mL
Saliva (pregnant female)	172– 2,225 pg/mL
Saliva (non-pregnant female)	Undetectable

Linearity of dilution

Linearity was determined using urine samples, one with a low estriol concentration (135.5 pg/mL) and one with a higher estriol concentration (6,729 pg/mL). The samples were mixed in the ratios given below. The measured estriol concentrations were compared to the expected values based on the ratios used.

High sample	Low sample	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
80%	20%	5,410	5,933	109.7
60%	40%	4,091	4,661	113.9
40%	60%	2,773	3,308	119.3
20%	80%	1,454	2,054	141.2
			Mean recovery	**121%**

Sensitivity

The analytical sensitivity of estriol is 5.33 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero and Std8 was assayed 20 times, and calculating the corresponding concentration.

Specificity

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

Steroid	Cross-reactivity %
Estriol	100
Estriol 3-glucuronide	57.16
Estriol 3-sulphate	38.50
16-Epiestriol	6.77
17β-Estradiol	0.03
17-Epiestriol	0.02
Androstenedione	<0.01
Androsterone	<0.01
Corticosterone	<0.01
Cortisol	<0.01
Cortisone	<0.01
Desoxycorticosterone	<0.01
Dehydroepiandrosterone (DHEA)	<0.01
Dehydroepiandrosterone-sulfate (DHEA-S)	<0.01
Dihydrotestosterone (DHT)	<0.01
17α-Estradiol	<0.01
Estrone	<0.01
Ethynylestradiol	<0.01
Progesterone	<0.01
Testosterone	<0.01

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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