

Estrone-3-Sulfate (E1S) Competitive ELISA Kit

Catalog Number EIA17E3S (96 tests)

Rev 1.0

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 Estrone-3-Sulfate (E1S) ELISA Kit is a solid-phase competitive Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of estrone-3-sulfate (E1S) in serum, EDTA and heparin plasma, urine, dried fecal extracts, and tissue culture media. The assay recognizes estrone-3-sulfate (E1S) 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 Estrone-3-Sulfate (E1S) Conjugate at -20°C or lower after opening.

Components	Quantity
Estrone-3-Sulfate (E1S) Standard; 40,000 pg/mL estrone-3-sulfate (E1S) in a special stabilizing solution	125 µL
Assay Buffer Concentrate (5X)	28 mL
Antibody Coated Wells, 96-well strip-well plate coated with goat anti-rabbit IgG	1 plate
Estrone-3-Sulfate (E1S) Antibody	3 mL
Estrone-3-Sulfate (E1S) Conjugate	3 mL
Dissociation Reagent	1 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)
- Plate shaker
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution

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 15 mL of Wash Solution Concentrate (20X) with 285 mL 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 14 mL of Assay Buffer (5X) with 56 mL 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.

For research use only. Not for use in diagnostic procedures.

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	<ol style="list-style-type: none"> 1. Dilute serum and plasma samples with an equal volume of the supplied Dissociation Reagent. 2. Dilute diluted samples $\geq 1:50$ with the 1X Assay Buffer prior to running in the assay for a final dilution $\geq 1:100$. <p>NOTE: Dissociation Reagent is to be used only with Serum and Plasma samples.</p>
Urine	<p>Dilute samples $\geq 1:8$ with 1X Assay Buffer.</p> <p>Note: A Urinary Creatinine Detection Kit (Cat. no. EIACUN) is available for measuring urine creatinine for normalization of estrone-3-sulfate (E1S) in a random urine specimens.</p>
Dried feces	<p>See detailed extraction protocol on the product page at thermofisher.com</p> <p>Note: The ethanol concentration in the final diluted Assay Buffer dilution added to the well should be $<1\%$.</p>
Tissue culture media	Perform sample dilutions with the corresponding tissue culture medium.

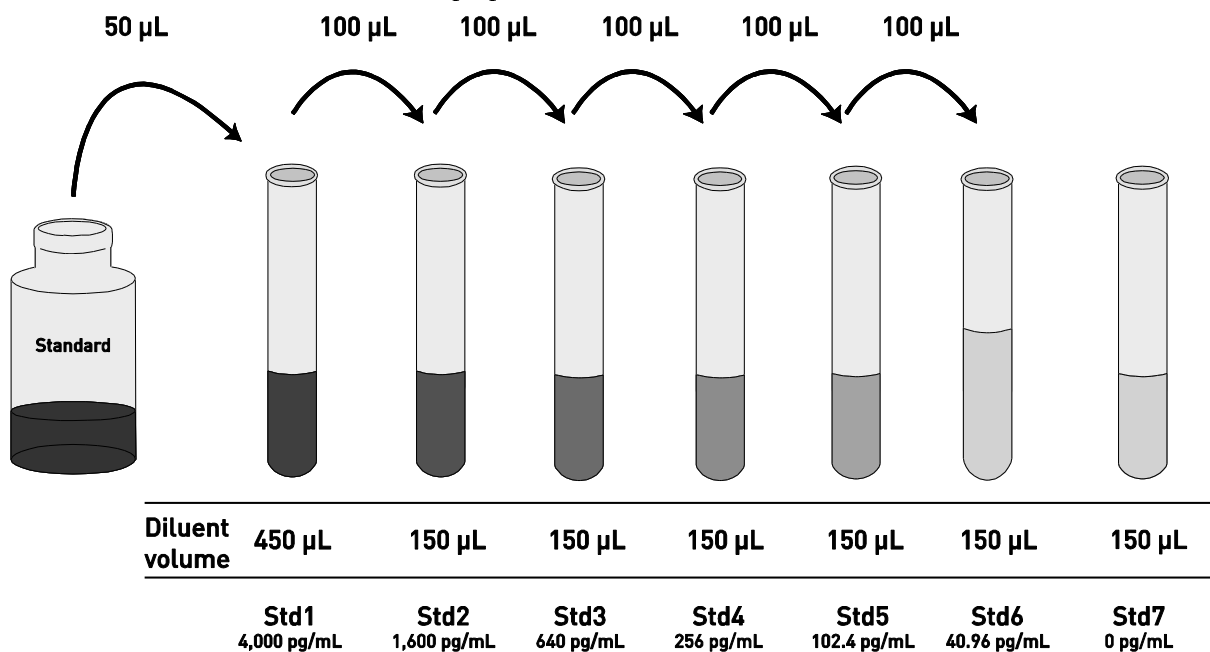
Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Instructions are for diluting standards from 4,000 to 40.96 pg/mL, but a curve can be obtained using a range of 1,600 to 40.96 pg/mL. Choose the range that fits your sample concentrations most appropriately.

The Estrone-3-Sulfate (E1S) 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 50 μL Estrone-3-Sulfate (E1S) Standard to one tube containing 450 μL 1X Assay Buffer and label as 4,000 pg/mL estrone-3-sulfate (E1S).
2. Add 150 μL 1X Assay Buffer to each of 6 tubes labeled as follows: 1,600; 640; 256; 102.4; 40.96; and 0 pg/mL estrone-3-sulfate (E1S).
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)

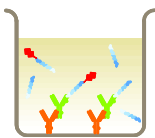
IMPORTANT! Perform a standard curve with each assay.

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

- Add 50 µL of standards or samples (see “Prepare samples” on page 2) to the appropriate wells.
- Add 75 µL of 1X Assay Buffer into wells for detecting non-specific binding (NSB).
- Add 25 µL of Estrone-3-Sulfate (E1S) Conjugate to each well.
- Add 25 µL of Estrone-3-Sulfate (E1S) Antibody to each well except NSB wells.
- Tap the side of the plate to mix. Cover the plate with plate sealer and incubate for 2 hours at room temperature with shaking.



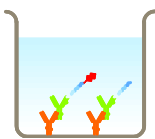
Note: If the plate is not shaken the bound of the signals will be ~35% lower.

- Thoroughly aspirate the solution and wash wells 4 times with 300 µL of 1X Wash Buffer.

Add chromogen

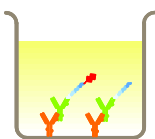
- Add 100 µL TMB Substrate to each well. The substrate solution will begin to turn blue.
- Incubate for 30 minutes at room temperature without shaking.

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

- Read the absorbance at 450 nm. Read the plate within 10 minutes¹⁵ after adding the Stop Solution.
- 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.
- Read the concentrations for unknown samples and controls from the standard 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 0–4,000 pg/mL estrone-3-sulfate (E1S).

Standard Estrone-3-Sulfate [E1S] (pg/mL)	Optical Density (450 nm)*
4,000	0.165
1,600	0.249
640	0.394
256	0.577
102.4	0.775
40.96	0.888
0	0.948

Note: The NSB gave a Mean OD value of 0.047.

Intra-assay precision

Samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	1,051.7	437.7	163.8
%CV	2.8	3.8	6.0

CV = Coefficient of Variation

Inter-assay precision

Samples were assayed in duplicates in 14 assay runs by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	1,025.4	459.9	158.0
%CV	8.1	9.4	8.1

CV = Coefficient of Variation

Performance characteristics, continued

Expected values

Five equine serum samples were tested in the assay at dilutions that ranged from 1:100 to 1:400 (1:2 with Dissociation Reagent followed by 1:50-1:200 with Assay Buffer). Adjusted neat concentrations of estrone-3-sulfate (E1S) in the serum ranged from 9.6 to 3,620 ng/mL.

Recovery

Recovery was determined by taking two equine serum samples treated with an equal volume of Dissociation Reagent and diluted \geq 1:50 with Assay Buffer, one with a low diluted estrone-3-sulfate (E1S) level of 69.4 pg/mL and one with a higher diluted level of 1,694.7 pg/mL, and mixing them in the ratios given below. The measured 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	1,369.6	1,252.3	91.4
60	40	1,044.6	1,108.0	106.1
40	60	719.5	731.8	101.7
20	80	394.5	441.4	111.9

Mean Recovery 102.8%

Specificity

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

Steroid	Cross-reactivity %
Estrone-3-sulfate	100
Estrone	267
Estrone-3-glucuronide	200
17 β -Estradiol	11.7
Estradiol-3-Glucuronide	5.7
Estradiol-3-Sulfate	5.0
Estradiol-17-Sulfate	0.2
Progesterone	<0.2
Estriol	<0.2
Cortisol	<0.2
Testosterone	<0.2
Pregnanediol	<0.2

Sensitivity

The analytical sensitivity of estrone-3-sulfate (E1S) is 26.4 pg/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.

Limited product warranty

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