KAMIYA BIOMEDICAL COMPANY

Estrone-3-Sulfate (E1S) EIA Kit

For the quantitative determination of E1S in dried fecal extracts, urine, serum/plasma and tissue culture media

Cat. No. KT-722

For Research Use Only.

PRODUCT INFORMATION
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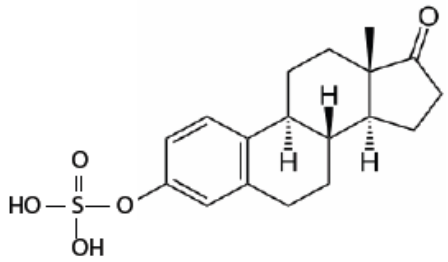
BACKGROUND
Estrone-3-sulfate, $C_{18}H_{22}O_5S$, (1, 3, 5(10)-Estratrien-3-ol-17-one sulfate, E1S) is synthesized in the fetal or cotyledonary portion of the placentome. Production rates of E1S are high in both males and females, with males producing 77 µg/day, and in women in early follicular phase, 95 µg/day and in early luteal phase, 182 µg/day. Estrone sulfate, present in plasma in a higher concentration than either unconjugated estrone or estradiol in nonpregnant women and normal men, appears to originate almost entirely from a conjugation of estrone and converted estradiolin nonglandular tissues. Estrone sulfate is only slowly cleared from plasma, thus its concentration does not fluctuate markedly during the day.

Estrone sulfate is quantitatively the most important circulating estrogen. In postmenopausal women with breast cancer, estrone sulfate concentrations in plasma have the same order of magnitude. Breast tumors contain sulfatase activity and can convert estrone sulfate into estradiol. Consequently, estrone sulfate provides a continuous supply of estrogens to hormone-responsive tumors.

Estrone-3-Sulfate, E1S



Cryptorchidism is a condition in which one or both testicles fail to descend into the scrotum, and it is considered to be a prevalent defect in horses. Bilaterally cryptorchid stallions do not produce viable spermatozoa but often exhibit normal secondary sexual characteristics such as libido, because of testosterone production by the interstitial cells of the retained testes. Bilateral cryptorchids, must be differentiated from geldings who exhibit stallion like behavior. Thus, the correct laboratory diagnosis of this condition is very important, especially when exploratory abdominal surgery is considered for the removal of retained testes. Several investigators have suggested measuring testosterone and estrone sulfate serum levels as reliable diagnostic aids for the condition.

PRINCIPLE
The Estrone-3-Sulfate (E1S) Immunoassay kit uses a specifically generated antibody to measure E1S in a variety of matrices, including serum, plasma, urine and fecal samples. The kit will quantitatively measure E1S present in diluted buffer samples and tissue culture media samples. Please read the complete kit insert before performing this assay. An E1S calibrator is provided to generate a calibration curve for the assay and all samples should be read off the calibration curve. Calibrators or diluted samples are pipetted into a clear microtiter plate coated with an antibody to capture rabbit antibodies. An E1S-peroxidase conjugate is added to the calibrators and samples in the wells. The binding reaction is initiated by the addition of a polyclonal antibody to E1S to each well. After a 2 hour incubation the plate is washed and substrate is added. The substrate reacts with the bound E1S-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 450nm wavelength. The concentration of the E1S in the sample is calculated, after making suitable correction for the dilution of the sample, using software available with most plate readers.

COMPONENTS
Coated Clear 96 Well Plates
Clear 1 by 8 break-apart strip well plastic microtiter plate(s) coated with goat anti-rabbit IgG.
1 Each

Estrone-3-Sulfate (E1S) Calibrator
Estrone-3-Sulfate (E1S) at 40,000 pg/mL in a special stabilizing solution.

125 µL

Estrone-3-Sulfate (E1S) Antibody
A rabbit polyclonal antibody specific for Estrone-3-Sulfate.
3 mL

Estrone-3-Sulfate (E1S) Conjugate
An Estrone-3-Sulfate-peroxidase conjugate in a special stabilizing solution.
3 mL

Assay Buffer Concentrate
A 5X concentrate that should be diluted with deionized or distilled water.
28 mL

Dissociation Reagent
1 mL
Dissociation Reagent is to be used only with Serum and Plasma samples.

Wash Buffer Concentrate
A 20X concentrate that should be diluted with deionized or distilled water.
30 mL

TMB Substrate
11 mL

Stop Solution
A 1M solution of hydrochloric acid. CAUSTIC.
5 mL

Plate Sealer
1 Each

STORAGE
All components of this kit should be stored at 4℃ until the expiration date of the kit.

OTHER MATERIALS REQUIRED
Distilled or deionized water.

Repeater pipet with disposable tips capable of dispensing 25 µL, 50 µL and 100 µL.

A microplate shaker.

Colorimetric 96 well microplate reader capable of reading optical density at 450 nm.

Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

PRECAUTIONS
As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

The antibody coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry. The silica gel pack will turn from blue to pink if the ziploc has not been closed properly.

This kit utilizes a peroxidase-based readout system. Buffers, including other manufacturers' Wash Buffers, containing sodium azide will inhibit color production from the enzyme. Make sure **all** buffers used for samples are **azide free**. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer.

The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

SAMPLE TYPES

This assay has been validated for serum, plasma, fecal, urine and tissue culture samples. Samples containing visible particulate should be centrifuged prior to using. Estrone-3-sulfate can be assayed in solid sample types.

Estrone-3-sulfate (E1S) is identical across all species and we expect this kit to measure estrone-3-sulfate from all sources. The end user should evaluate recoveries of E1S in other sample matrices being tested.

SAMPLE PREPARATION

Serum and Plasma Samples
Serum and plasma samples should be diluted with an equal volume of the supplied Dissociation Reagent.
The diluted samples should then be further diluted ≥ 1:50 with the supplied Assay Buffer prior running in the assay.
NOTE: Dissociation Reagent is to be used only with Serum and Plasma samples.

Dried Fecal Samples
The ethanol concentration in the final Assay Buffer dilution added to the well should be <1%.

Urine Samples
Urine samples should be diluted at least 1:8 times with the provided Assay Buffer.

Tissue Culture Media
For measuring estrone-3-sulfate in tissue culture media (TCM), samples should be read off a calibration curve generated in TCM. Samples may need to be diluted further in TCM.

Use all samples within 2 hours of preparation.

REAGENT PREPARATION

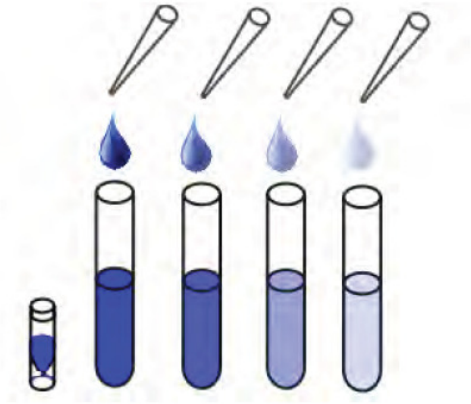
Allow the kit reagents to come to room temperature for 30 minutes. We recommend that all calibrators and samples be run in duplicate to allow the end user to accurately determine E1S concentrations. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

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℃ for 3 months.

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 at room temperature for 3 months.

Calibrator Preparation
Label six test tubes as #1 through #6. Pipet 450 µL of Assay Buffer into tube #1 and 150 µL into tubes #2 to #6. **The Estrone-3-Sulfate stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery.** Carefully add 50 µL of the estrone-3-sulfate stock solution to tube #1 and vortex completely. Take 100 µL of the estrone-3-sulfate solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #6. The concentration of estrone-3-sulfate in tubes 1 through 6 will be 4,000, 1,600, 640, 256, 102.4, and 40.96 pg/mL.

Use all Calibrators within 2 hours of preparation.



	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Assay Buffer (µL)	**450**	150	150	150	150	150
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5
Vol of Addition (µL)	**50**	100	100	100	100	100
Final Conc (pg/mL)	4,000	1,600	640	256	102.4	40.96

ASSAY PROTOCOL

1. Use the plate layout sheet on the back page to aid in proper sample and calibrator identification. Determine the number of wells to be used and return unused wells to the foil pouch with desiccant. Seal the ziploc plate bag and store at 4˚C.

2. Pipet 50 µL of samples or calibrators into wells in the plate.

3. Pipet 75 µL of Assay Buffer into the non-specific binding (NSB) wells.

4. Pipet 50 µL of Assay Buffer into wells to act as maximum binding wells (Bo or 0 pg/mL).

5. Add 25 µL of the Estrone-3-Sulfate Conjugate to each well using a repeater pipet.

6. Add 25 µL of the Estrone-3-Sulfate Antibody to each well, **except the NSB wells**, using a repeater pipet.

7. 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 35% lower.

8. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.

9. Add 100 µL of the TMB Substrate to each well, using a repeater pipet.

10. Incubate the plate at room temperature for 30 minutes without shaking.

11. Add 50 µL of the Stop Solution to each well, using a repeater pipet.

12. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.

13. Use the plate reader's built-in 4PLC software capabilities to calculate estrone-3- sulfate (E1S) concentration for each sample.

CALCULATION OF RESULTS

Average the duplicate OD readings for each calibrator and sample. Create a calibration curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean OD's for the NSB. The sample concentrations obtained, calculated from the %B/B0 curve, should be multiplied by the dilution factor to obtain neat sample values.

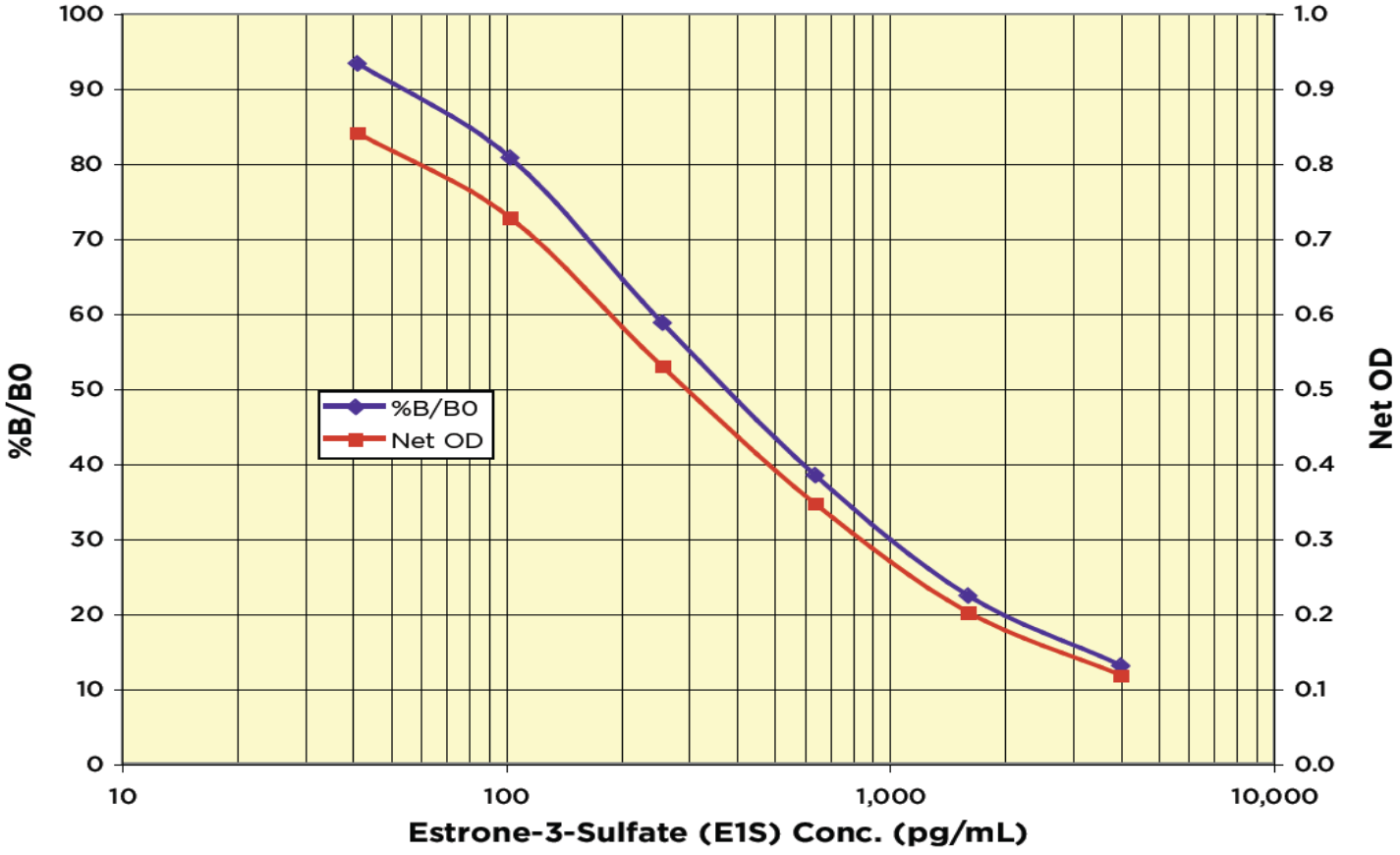
TYPICAL DATA

Sample	Mean OD	Net OD	% B/B0	E1S Conc. (pg/mL)
NSB	0.047	0.000	-	-
Standard 1	0.165	0.118	13.1	4,000
Standard 2	0.249	0.202	22.4	1,600
Standard 3	0.394	0.347	38.5	640
Standard 4	0.577	0.530	58.8	256
Standard 5	0.775	0.728	80.8	102.4
Standard 6	0.888	0.841	93.3	40.96
B0	0.948	0.901	100.0	0
Sample 1	0.475	0.428	47.5	423.9
Sample 2	0.678	0.631	70.0	165.5

Always run your own calibration curve for calculation of results. Do not use this data.

Conversion Factor: 100 pg/mL of E1S is equivalent to 268.5 pM.

Typical Calibration Curves



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VALIDATION DATA

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the OD's for twenty wells run for each of the B0 and calibrator #6. The detection limit was determined at two (2) standard deviations from the B0 along the calibration curve.

Sensitivity was determined as 26.4 pg/mL.

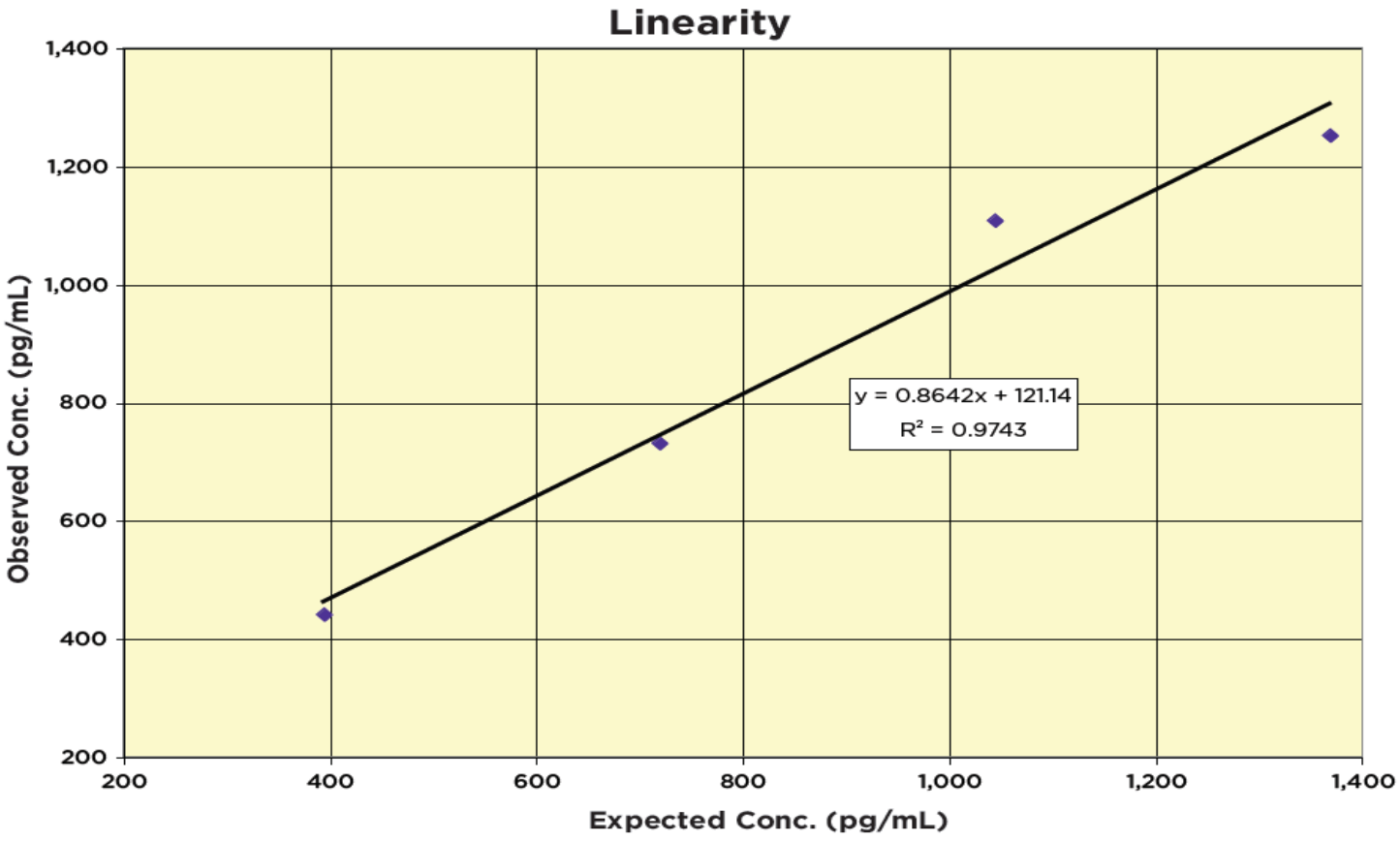
The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty runs for each of the zero calibrator and a low concentration equine serum sample.

Limit of Detection was determined as 54.6 pg/mL

Linearity

Linearity was determined by taking two equine serum samples treated with an equal volume of Dissociation Reagent and diluted ≥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 Serum	Low Serum	Observed Conc. (pg/mL)	Expected Conc. (pg/mL)	% Recovery
80%	20%	1,252.3	1,369.6	91.4
60%	40%	1,108.0	1,044.6	106.1
40%	60%	731.8	719.5	101.7
20%	80%	441.4	394.5	111.9
			Mean Recovery	**102.8%**



Intra Assay Precision
Three serum samples treated with Dissociation Reagent and diluted with Assay Buffer were run in replicates of 20 in an assay. The mean and precision of the calculated estrone-3-sulfate (E1S) concentrations were:

Sample	E1S Conc. (pg/mL)	%CV
1	1,051.7	2.8
2	437.7	3.8
3	163.8	6.0

Inter Assay Precision
Three serum samples treated with Dissociation Reagent and diluted with Assay Buffer were run in duplicates in fourteen assays run over multiple days by three operators. The mean and precision of the calculated estrone-3-sulfate (E1S) concentrations were:

Sample	E1S Conc. (pg/mL)	%CV
1	1,025.4	8.1
2	459.9	9.4
3	158.0	8.1

SAMPLE 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.

CROSS REACTIVITY
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%
17ß-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%

FOR RESEARCH USE ONLY

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