

ARBOR ASSAYS™  
Interactive Assay Solutions™



# DetectX<sup>®</sup>

## Estrone-3-Sulfate (E1S) Enzyme Immunoassay Kit

1 Plate Kit Catalog Number K038-H1

5 Plate Kit Catalog Number K038-H5

Species Independent

### Sample Types Validated:

**Dried Fecal Extracts, Urine,  
Serum/Plasma, and Tissue Culture Media**

Please read this insert completely prior to using the product.  
For research use only. Not for use in diagnostic procedures.

[www.ArborAssays.com](http://www.ArborAssays.com)   

**K038-H WEB 190502**

# TABLE OF CONTENTS

|  |       |
|--|-------|
| Background                                   | 3     |
| Assay Principle                              | 4     |
| Related Products                             | 4     |
| Supplied Components                          | 5     |
| Storage Instructions                         | 5     |
| Other Materials Required                     | 6     |
| Precautions                                  | 6     |
| Sample Types                                 | 7     |
| Sample Preparation                           | 7     |
| Reagent Preparation                          | 8     |
| Assay Protocol                               | 9     |
| Calculation of Results                       | 10    |
| Typical Data                                 | 10-11 |
| Validation Data Sensitivity, Linearity, etc. | 11-13 |
| Samples Values and Cross Reactivity          | 14    |
| Warranty & Contact Information               | 15    |
| Plate Layout Sheet                           | 16    |

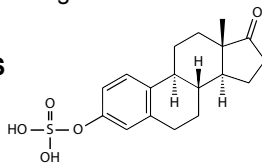


## BACKGROUND

Estrone-3-sulfate, C<sub>18</sub>H<sub>22</sub>O<sub>5</sub>S, (1, 3, 5(10)-Estratrien-3-ol-17-one sulfate, E1S) is synthesized in the fetal or cotyledonary portion of the placenta<sup>1</sup>. 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<sup>2</sup>. 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 estradiol in nonglandular tissues<sup>3</sup>. Estrone sulfate is only slowly cleared from plasma, thus its concentration does not fluctuate markedly during the day<sup>4,5</sup>.

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<sup>6</sup> and can convert estrone sulfate into estradiol<sup>7</sup>. 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<sup>8,9</sup>. 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<sup>8,9</sup>.

1. Hoffmann, B, Wagner, WC, Hoxon, JE, Bahr, J., Observations concerning the functional status of the corpus luteum and the placenta around parturition. *Anom. Reprod. Sci.*, 1979, 2:253-266.
2. Ruder, HJ, Lorlaux, L., and Lipsett, MB. Estrone Sulfate: Production Rate and Metabolism in Man. 1972, *J. Clin. Invest.*, 51:1020-1033.
3. Longcope C. The metabolism of estrone sulfate in normal males. 1972, *J Clin Endocrinol* 34:113-122.
4. Wright K, Collins DC, Musey P1, Preedy JRK. A specific radioimmunoassay for estrone sulfate in plasma and urine without hydrolysis. 1978, *J Clin Endocrinol Metab* 47, 1092-1098.
5. Hawkins RA, Oakey RE. Estimation of oestrone sulphate, oestradiol-17 and oestrone in peripheral plasma: concentrations during the menstrual cycle and in men. 1974, *J Endocrinol* 60, 3-17.
6. Dao LD, Hayes C, Libby, PR. Steroid sulfatase activities in human breast tumors. *Proc Soc Exp Biol Med* 1974, 146:381-384.
7. Wilking N, Carlstrom WK, Gustafsson SA, et al. Oestrogen receptors and metabolism of oestrone sulphate in human mammary carcinoma. 1980, *Eur J Cancer* 16, 1339-1334.
8. Arighi M, Bosu WTK. Comparison of hormonal methods for diagnosis of cryptorchidism in horses. *J. Equine Vet. Sci.* 1989, 9:20-26.
9. Liepold HW, DeBowes RM, Bennett S, et al. Cryptorchidism in the horse—genetic implications, in *Proceedings. 31st Ann. Conv. Am. Assoc. Equine Practn.* 1985;579.

## ASSAY PRINCIPLE

The DetectX® Estrone-3-Glucuronide (E1G) Immunoassay Kit uses a specifically generated antibody to measure E1G and its metabolites in urine and fecal samples, or in extracted serum and plasma. This kit is not recommended for serum, plasma, or saliva samples without extraction. The kit will quantitatively measure E1G present in diluted buffer samples and tissue culture media samples. Please read the complete kit insert before performing this assay. An E1G standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Standards or diluted samples are pipetted into a clear microtiter plate coated with an antibody to capture rabbit antibodies. Two formats can be chosen. Using 50 µL of sample or standards provides a standard curve from 1,000 to 15.625 pg/mL. A 100 µL standard and sample volume format will give a standard curve from x to y pg/mL. An E1G-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 E1G to each well. After a 2 hour incubation the plate is washed and substrate is added. The substrate reacts with the bound E1G-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. The concentration of the E1G in the sample is calculated, after making suitable correction for the dilution of the sample, using software available with most plate readers.

## RELATED PRODUCTS

| Kits   | Catalog No.            |
|--|------------------------|
| <a href="#">17-Hydroxyprogesterone EIA Kits</a>                                    | K053-H1/H5             |
| <a href="#">Aldosterone EIA &amp; CLIA Kits</a>                                    | K052-H1/H5, K052-C1/C5 |
| <a href="#">Allopregnanolone EIA &amp; CLIA Kits</a>                               | K044-H1/H5, K044-C1/C5 |
| <a href="#">Ceruloplasmin Colorimetric Activity Kit</a>                            | K035-H1                |
| <a href="#">Dehydro-epiandrosterone sulfate (DHEA-S) EIA Kits</a>                  | K054-H1/H5             |
| <a href="#">Estradiol Non-Invasive &amp; Serum EIA Kits</a>                        | K030-H1/H5, KB30-H1/H5 |
| <a href="#">Estrone EIA Kits</a>   | K031-H1/H5             |
| <a href="#">Estrone-3-Sulfate (E1S) EIA Kits</a>                                   | K038-H1/H5             |
| <a href="#">Levonorgestrel EIA Kits</a>  | K058-H1/H5             |
| <a href="#">Oxytocin EIA &amp; CLIA Kits</a>                                       | K048-H1/H5             |
| <a href="#">PGFM (13,14-dihydro-15-keto-Prostaglandin F<sub>2α</sub>) EIA Kits</a> | K022-H1/H5             |
| <a href="#">Pregnanediol Glucuronide (PDG) EIA Kits</a>                            | K037-H1/H5             |
| <a href="#">Progesterone EIA Kits</a>  | K025-H1/H5             |
| <a href="#">Prolactin EIA Kit</a>  | K040-H1                |
| <a href="#">Testosterone EIA Kits</a>  | K032-H1/H5             |
| <a href="#">Urinary Creatinine Detection Kit (2 or 10 Plates)</a>                  | K002-H1/H5             |



## SUPPLIED COMPONENTS

### Coated Clear 96 Well Plates

Clear 1 by 8 break-apart strip well plastic microtiter plate(s) coated with goat anti-rabbit IgG.  
Kit K038-H1 or -H5                      1 or 5 Each                      Catalog Number X016-1EA

### Estrone-3-Sulfate (E1S) Standard

Estrone-3-Sulfate (E1S) at 40,000 pg/mL in a special stabilizing solution.  
Kit K038-H1 or -H5                      125 µL or 625 µL                      Catalog Number C135-125UL or -625UL

### DetectX® Estrone-3-Sulfate (E1S) Antibody

A rabbit polyclonal antibody specific for Estrone-3-Sulfate.  
Kit K038-H1 or -H5                      3 mL or 13 mL                      Catalog Number C133-3ML or -13ML

### DetectX® Estrone-3-Sulfate (E1S) Conjugate [Must be stored at -20°C.](#)

An Estrone-3-Sulfate-peroxidase conjugate in a special stabilizing solution.  
Kit K038-H1 or -H5                      3 mL or 13 mL                      Catalog Number C134-3ML or -13ML

### Assay Buffer Concentrate

A 5X concentrate that should be diluted with deionized or distilled water.  
Kit K038-H1 or -H5                      28 mL or 55 mL                      Catalog Number X065-28ML or -55 ML

### Dissociation Reagent

Dissociation Reagent is to be used only with Serum and Plasma samples.  
Kit K038-H1 or -H5                      1 mL or 5 mL                      Catalog Number X017-1ML or -5ML

### Wash Buffer Concentrate

A 20X concentrate that should be diluted with deionized or distilled water.  
Kit K038-H1 or -H5                      30 mL or 125 mL                      Catalog Number X007-30ML or -125ML

### TMB Substrate

Kit K038-H1 or -H5                      11 mL or 55 mL                      Catalog Number X019-11ML or -55ML

### Stop Solution

A 1M solution of hydrochloric acid. **CAUSTIC.**  
Kit K038-H1 or -H5                      5 mL or 25 mL                      Catalog Number X020-5ML or -25ML

### Plate Sealer

Kit K038-H1 or -H5                      1 or 5 Each                      Catalog Number X002-1EA

## STORAGE INSTRUCTIONS

The unopened kit must be stored at -20°C. Once opened the kit can be stored at 4°C up to the expiration date on the kit label, **except for the [Estrone-3-Sulfate \(E1S\) Conjugate](#). This must be stored at -20°C.**

## OTHER MATERIALS REQUIRED

Distilled or deionized water.

Repeater pipet with disposable tips capable of dispensing 25, 50, and 100  $\mu$ L.

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 as prepared on Page 8.

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



ARBOR  
ASSAYS

## 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 by using one of the extraction protocols available on our website at: [www.arborassays.com/resources/#protocols](http://www.arborassays.com/resources/#protocols).

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

We have a detailed Extraction Protocol available on our website at: [www.arborassays.com/resources/#protocols](http://www.arborassays.com/resources/#protocols). 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 with the provided Assay Buffer. For comparison to creatinine as a urine volume marker please see our NIST-calibrated 2 plate and 10 plate Urinary Creatinine Detection kits, K002-H1 and K002-H5.

### Tissue Culture Media

For measuring estrone-3-sulfate 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.

**Use all samples within 2 hours of preparation.**

## REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

### Assay Buffer

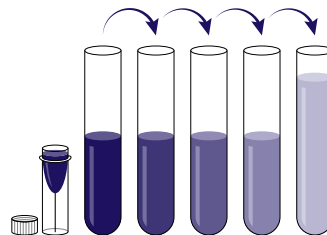
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

### 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.

### Standard Preparation

Label test tubes as #1 through #6. Pipet 450  $\mu\text{L}$  of Assay Buffer into tube #1 and 150  $\mu\text{L}$  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  $\mu\text{L}$  of the estrone-3-sulfate stock solution to tube #1 and vortex completely. Take 100  $\mu\text{L}$  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  $\text{pg/mL}$ .



Use all Standards within 2 hours of preparation.

|                                   | Std 1 | Std 2 | Std 3 | Std 4 | Std 5 | Std 6 |
|-----------------------------------|-------|-------|-------|-------|-------|-------|
| Assay Buffer ( $\mu\text{L}$ )    | 450   | 150   | 150   | 150   | 150   | 150   |
| Addition                          | Stock | Std 1 | Std 2 | Std 3 | Std 4 | Std 5 |
| Vol of Addition ( $\mu\text{L}$ ) | 50    | 100   | 100   | 100   | 100   | 100   |
| Final Conc ( $\text{pg/mL}$ )     | 4,000 | 1,600 | 640   | 256   | 102.4 | 40.96 |



## ASSAY PROTOCOL

**We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine E1S concentrations.**

1. Use the plate layout sheet on the back page to aid in proper sample and standard 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 standards 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 the maximum binding (B0 or Zero standard) wells.
5. Add 25 µL of the DetectX® Estrone-3-Sulfate Conjugate to each well using a repeater pipet.
6. Add 25 µL of the DetectX® 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.

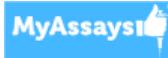
*NOTE: If you are using only part of a strip well plate, at the end of the assay throw away the used wells and retain the plate frame for use with the remaining unused wells.*

## CALCULATION OF RESULTS

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 NSB. The sample concentrations obtained, calculated from the %B/B0 curve, should be multiplied by the dilution factor to obtain neat sample values.

Or use the online tool from MyAssays to calculate the data:

[www.myassays.com/arbor-assays-estrone-3-sulfate-\(e1s\)-eia-kit.assay](http://www.myassays.com/arbor-assays-estrone-3-sulfate-(e1s)-eia-kit.assay)



### 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 standard curve for calculation of results. Do not use this data.**

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



\*The MyAssays logo is a registered trademark of MyAssays Ltd.

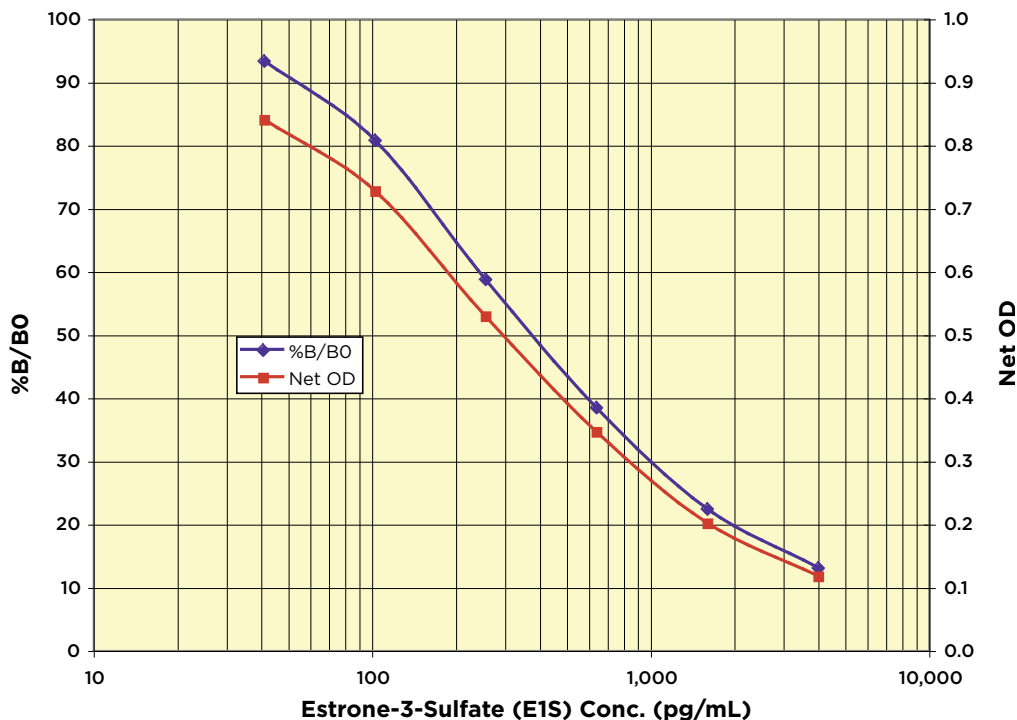
ARBOR  
ASSAYS

10

K038-H WEB 190502

EXPECT ASSAY ARTISTRY

## Typical Standard Curves



Always run your own standard curves for calculation of results. Do not use this data.

## 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 standard #6. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve. **Sensitivity was determined as 26.4 pg/mL.**

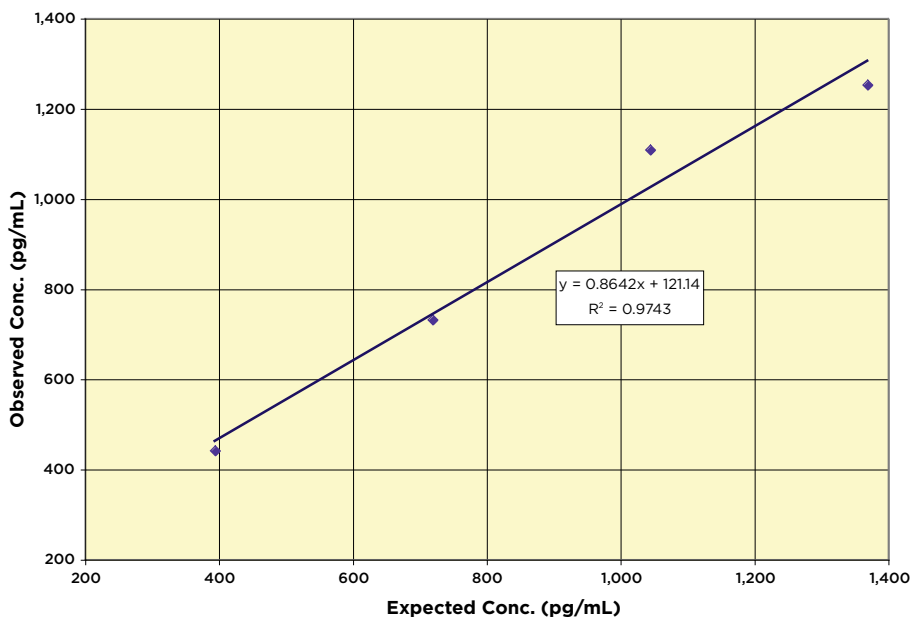
The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty runs for each of the zero standard and a low concentration equine serum sample. **Limit of Detection was determined as 54.6 pg/mL.**

## Linearity

Linearity was determined by taking two equine serum samples treated with an equal volume of Dissociation Reagent and diluted  $\geq 1:50$  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 Serum           | Low Serum | Expected Conc.<br>(pg/mL) | Observed Conc.<br>(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         |
| <b>Mean Recovery</b> |           |                           |                           | <b>102.8%</b> |

## Linearity



### 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%                 |
| 17 $\beta$ -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%               |

## LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

## CONTACT INFORMATION

For details concerning this kit or to order any of our products please contact us:

### Arbor Assays

1514 Eisenhower Place  
Ann Arbor, Michigan 48108 USA

Phone: 734-677-1774

Fax: 734-677-6860

Web: [www.ArborAssays.com](http://www.ArborAssays.com)

### Email Addresses:

[Info@ArborAssays.com](mailto:Info@ArborAssays.com)

[Orders@ArborAssays.com](mailto:Orders@ArborAssays.com)

[Technical@ArborAssays.com](mailto:Technical@ArborAssays.com)

[Contracts@ArborAssays.com](mailto:Contracts@ArborAssays.com)



## OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with EIA kits for wildlife conservation research.

*DetectX<sup>®</sup>, ThioStar<sup>®</sup> and the Arbor Assays logo are all registered trademarks.*

[www.ArborAssays.com](http://www.ArborAssays.com)

15

K038-H WEB 190502



ARBOR  
ASSAYS

| H | G | F | E | D | C | B | A |    |
|---|---|---|---|---|---|---|---|----|
|   |   |   |   |   |   |   |   | 1  |
|   |   |   |   |   |   |   |   | 2  |
|   |   |   |   |   |   |   |   | 3  |
|   |   |   |   |   |   |   |   | 4  |
|   |   |   |   |   |   |   |   | 5  |
|   |   |   |   |   |   |   |   | 6  |
|   |   |   |   |   |   |   |   | 7  |
|   |   |   |   |   |   |   |   | 8  |
|   |   |   |   |   |   |   |   | 9  |
|   |   |   |   |   |   |   |   | 10 |
|   |   |   |   |   |   |   |   | 11 |
|   |   |   |   |   |   |   |   | 12 |



Printed on Forest Stewardship Council certified paper