Equine Estrone 3-Sulfate ELISA Kit

Enzyme Immunoassay for the quantification of Horse (Equine) Estrone-3-Sulfate in serum.

Catalog number: ARG80653

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INTRODUCTION

Estrone-3-Sulfate (E3S) is the predominant conjugated estrogen during pregnancy. It is produced by the fetus, possibly in association with the endometrium in the pregnant mare.

Different hormones are important for the complex events that occur during pregnancy in all mammals. In the mare these events include the maintenance of the corpus luteum function, formation of endometrial cups and development of secundary corpora lutea. Progesterone and PMSG (Pregnant Mare Serum Gonadotropine, eCG) and also free Estrogens, e.g. Estrone, are associated with these processes. It has been shown, that Estrone is rapidly conjugated after secretion and the ratio between conjugated and unconjugated estrogens is 100:1 in mare serum.

The conjugated estrogenes, especially Estrone-3-sulfate, provide the opportunity to improve the accu- racy of pregnancy diagnosis, to monitor the pregnancy and to distinguish whether the fetal development is normal or impaired. The diagnosis of embryonic death is usually made by using techniques of palpation of the uterus per rectum or ultrasound echography. The determination of Estrone-3-sulfate is an aid in the non-invasive diagnosis which allows a monitoring of the feto-placental unit during pregnancy. Only in mares with normal fetal development the values of Estrone-3-sulfate show a tremendous increase between day 75 and 100 of gestation.

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. An antibody specific for Estrone-3-Sulfate has been pre-coated onto a microtiter plate. Endogenous Estrone-3-Sulfate of a sample competes with a biotin conjugated Estrone-3-Sulfate for binding to the coated antibody on the plate. Afterwards, horseradish peroxidase-labeled streptavidine is added. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of Estrone-3-Sulfate in the sample. After addition of the substrate solution, the intensity of color developed is inversely proportional to the concentration of Estrone-3-Sulfate in the sample. Estrone-3-Sulfate concentration in the sample is calculated through a calibration curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	1 plate	4°C
Calibrators A-G	7 vials	4°C, lyophilized
Biotin-Labeled Estrone-3-Sulfate	11 ml (ready to use)	4°C
HRP conjugated Streptavidine	3 ml (ready to use)	4°C
Equine E3S Sample Buffer	11 ml (ready to use)	4°C
10X Wash buffer	50 ml	4°C
TMB substrate	22 ml (ready to use)	4°C (Protect from light)
STOP solution	7 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 10X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer.
- Calibrators: Reconstitute lyophilized Calibrator A with 2.0 ml distilled water and Calibrator B through Calibrator G with 1.0 ml distilled water 30 min. before use.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 20 µl of standards, controls and samples in duplicate into sample wells.
3. Add 100 µl of Biotin-Labeled Estrone-3-Sulfate into each well.
4. Add 100 µl of Sample Buffer into each well. Incubate for 1 hours at RT.
5. Add 25 µl of HRP-Streptavidine into each well.
6. Incubate for 30 minutes at RT.
7. Aspirate each well and wash, repeating the process 3 times for a total 4 washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
8. Add 200 µl of TMB mixture to each well. Incubate for 30 minutes at room temperature in dark.
9. Add 50 µl of Stop Solution to each well.
10. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

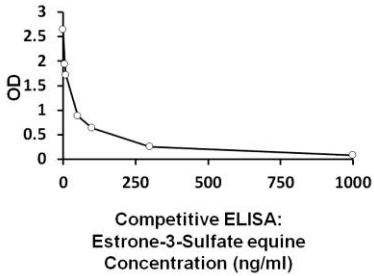
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Specificity

Compound	Amount added ng/ml	Apparent Conc. ng/ml	Percent Crossreactivity (%)
Estrone	1000	78	7.8
Estradiol	1000	2.2	0.22
Estradiol-sulfate	1000	4.5	0.45
Equilin	1000	8.8	0.88
Equilenin	1000	2.3	0.23
Equilin-sulfate	1000	27.2	2.7
Equilenin-Sulfate	1000	217	21.7
Androstendione	1000	1.0	0.1
Dehydro-iso-androsterone-3-sulfate	1000	5.2	0.5
Dihydrotestosterone	1000	2.0	0.2
Testosterone	1000	1.4	0.14
Pregnolone	1000	2.3	0.23
Androsterone	1000	1.5	0.15

Sensitivity

The detection limit of the assay, defined as the concentration three standard deviation above the response at zero dose, is approximately 0.14 ng/ml.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 7.2% and inter-assay precision was 7.3%.

Recovery

81-107%