Bowei-biotech
for better bioassays

Specific Estrone-3-Sulfate (E1S) ELISA

For Research Use Only
Cat. No.: BW-E1S ELISA

1. Introduction

Determination of E1S in biological samples of female herbivores has long been used in livestock for pregnancy detection and litter size classification.

When being used as a marker for disease diagnosis in human populations, E1S is also useful for various of clinical settings [1], e.g., for assessing the risk stratification of breast cancer [2], monitoring of the proliferation promotion of endometrial cancer [3], providing prognostic information in advanced prostatic carcinoma [4], and monitoring of the response to certain hormonal therapy for malignancy [5]. A lot of immunoassays have been designed for E1S determination, however, the utility of these assays for human sample analysis are very limited, mainly due to their cross-reactivity with dehydroepiandrosterone sulphate (DHEAS) [6]. A very slight cross-reactivity with DHEAS in E1S immunoassay will lead to unacceptable bias in view of the 10- to 10000-fold excess of DHEAS over E1S in male serum [4]. Besides DHEAS, other steroids may also exert interferences on E1S immunoassay.

This assay is designed to specific determination of E1S concentration by using an anti-E1S McAb which show < 0.0001% cross-reactivity with DHEAS; also, it is direct assay without the need for sample pretreatment.

2. Principle of the Assay

The E1S ELISA adopts a typical competitive immunoassay principle.
E1S present in samples, calibrators, and controls competes with E1S coated on the microplate wells for binding a limited number of anti-E1S monoclonal antibody. Unbound components are removed by washing. Horseradish peroxidase (HRP) conjugated secondary antibody is added to trace the bound primary antibody. After a final wash, TMB substrate is added to produce a color reaction. The absorbance intensity is inversely proportional to the E1S concentration in the sample. A calibration curve is established using calibrators to calculate E1S concentrations in unknown samples.

3. Package Contents

Kit contents are sufficient for 96 tests (8×12 wells).
Expiry date is labeled on the outer box.

Components	Specification	Description
① E1S Coated Plate	8×12 wells, detachable	Microwells precoated with E1S antigen
② Primary Antibody	1 bottle (0.5mL, 25×)	Monoclonal antibody against E1S
③ Assay Buffer	1 bottle (30mL)	PBS buffer containing BSA and Tween20
④ Secondary Antibody	1 bottle (0.5mL, 25×)	Sheep antimouse IgG- HRP conjugate
⑤ TMB Chromogenic Substrate	1 bottle (15mL)	Single- component TMB for color development
⑥ Wash Buffer	2 bottles (25mL/bottle, 10×)	0.05% Tween20 in PBS
⑦ Stop Solution	1 bottle (15mL)	1 M Citric acid solution
⑧ E1S Calibrators	6 vials (1mL each)	0, 0.2, 0.8, 2, 5, 10ng/mL

4. Kit Storage and Stability

- Store the entire kit at 2–8°C.
- Unused plate strips must be kept in the aluminum foil bag with desiccant; keep sealed and dry.
- Shelf life: 12 months from production.
- Use opened reagents and plate strips within 3 months.

5. Equipment and Materials Required but not Provided

- Single-channel micropipettes (10–100µL) and disposable tips
- 8/12-channel micropipette (50–200µL)
- Orbital microplate shaker
- Microplate reader (detection wavelength: 450nm)
- Distilled or deionized water
- Microplate washer (optional)

6. Sample Collection and Storage

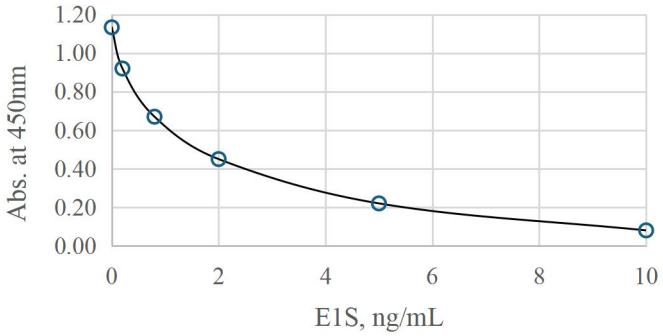
6.1 The test can be performed with serum as well as with EDTA plasma. The applicability for analyzing specimens other than plasma or serum remains to be verified.
6.2 Hemolytic and lipemic samples should not be used.
6.3 The samples can be stored up to 15 days at 2-8°C. For a longer storage (up to 18 months) the samples need to be frozen at -20°C. Repeated freezing and thawing should be avoided.
6.4 Hemolytic, lipemic and icteric specimens should not be used.

7. Experimental Procedure

7.1 Separate the strips to be used by cutting through the protective tape with a sharp knife and transfer them to a strip frame. Pull off the protective tape from the strips intended for use. Return the remaining strips to the plastic tray pack and reseal.
7.2 According to the number of samples to be tested, add the Primary Antibody to the required volume of Assay Buffer at a ratio of 1:25, and mix thoroughly. This should be done within 60 minutes before use.
7.3 Pipette 25µL of the E1S calibrator and samples into the bottom of each well.
7.4 Add 75µL of Primary Antibody solution to each well, incubate the frame for 90 minutes at room temperature on an orbital shaker at medium speed.
7.5 Discard or aspirate the contents of the wells and wash thoroughly with 300µl wash buffer diluted 10 times with distilled water, remove residual liquid by tapping the inverted plate on a clean absorbent paper. Repeat this 3 times. Alternatively, a Microplate washer may be used.
7.6 Within 60 minutes before use, prepare the needed volume of Secondary Antibody solution by mixing 1.5mL Assay Buffer with 60µL Secondary Antibody stock solution (25×) per strip.
7.7 Dispense 100µL of Secondary Antibody solution into each well and incubate the frame for 60 minutes at room temperature on an orbital shaker at medium speed.
7.8 After incubation, wash the wells as in step 7.5. Ensure no residual wash buffer remains.
7.9 Dispense 100µL of Chromogenic Substrate solution into each well, and mix thoroughly. Incubate for 20 minutes at room temperature with slow shaking.
7.10 Add 100µL of Stop Solution into each well. Shake for at least 10 seconds on an orbital shaker.
7.11 Quantitation. Read the optical density at 450nm in a microplate photometer within 10 minutes. Plot absorbance vs. calibrator concentration to construct the E1S calibrator curve. When using analyzing software, it is recommended to use the Four Parameter Logistic (4PL) Regression (alternatively: cubic-spline or logit-log). The concentration of the controls and samples in ng/ml can be read directly from this calibration curve by using their average optical density.

8. Performance Characteristics

8.1 The typical standard curve for the E1S-ELISA is shown below.



8.2 Analytical sensitivity. The analytical sensitivity of the E1S ELISA is typically better than 0.05 ng/mL, defined as the mean of the measurement values for the zero calibrator minus 2 standard deviations (mean value - 2SD).
8.3 Recovery. Spiked serum samples were prepared by adding varying levels of E1S to pooled serum specimens containing a known amount of E1S. Recoveries were in the range of 92- 101% with a mean value of 95.2% (n=15).
8.4 The cross reactivities (at the 50 % displacement level) with the steroids of similar structure were listed below.

Substance	Cross Reactivity (%)
ES	100
Estrone (E1)	0.02
DHEAS	0.00012%
Progesterone	<0.01
Testosterone	<0.01
Estrone glucuronide	<0.01
Estradiol (E2)	<0.01
17b-Estradiol 3-sulfate	<0.01

8.5 Intra- assay precision: ≤ 10%, Inter- assay precision: ≤ 15%.
8.6 The range of measurement: 0.1- 10ng/mL.

9. Notes

- This kit is for research use only, not for human diagnostic or therapeutic procedures.
- Avoid repeated freezing and thawing of reagents.
- Protect TMB substrate from light and avoid contact with metal ions.
- Strictly follow incubation time and temperature to ensure reproducibility.
- If the OD value of the sample exceeds the range of the calibration curve, the sample needs to be diluted with the zero-concentration calibrator for detection to ensure that the result is within the linear range.

10. References

1) Atoosa Rezvanpour & Andrew C. Don-Wauchope. Clinical implications of estrone sulfate measurement in laboratory medicine. Crit Rev Clin Lab Sci, 2017, 54, 73–86.
2) James RE, Lukanova A, Dossus L, et al. Postmenopausal serum sex steroids and risk of hormone receptor-positive and -negative breast cancer: a nested case-control study. Cancer Prev Res 2011, 4, 1626–35.
3) Lukanova A, Lundin E, Micheli A, et al. Circulating levels of sex steroid hormones and risk of endometrial cancer in postmenopausal women. Int J Cancer 2004, 108, 425–32.
4) Specific radioimmunoassay of estrone sulfate: Application to measurement in male plasma.

Journal of Steroid Biochemistry & Molecular Biology 2002, 81, 85–94.
5) Farhat GN, Parimi N, Chlebowski RT, et al. Sex hormone levels and risk of breast cancer with estrogen plus progestin. J Natl Cancer Inst., 2013, 105, 1496–503.
6) 6. Frank Giton, Patrick Caron, René Bérubé, et al. Plasma estrone sulfate assay in men: Comparison of radioimmunoassay, mass spectrometry coupled to gas chromatography (GC–MS), and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Clinica Chimica Acta, 2010, 411, 1208–1213.

Developed and Manufactured by:

Shanghai Baiwei Biotechnology Co., Ltd.

Address: Unit C401-3, Building 7, Pujiang Wisdom Valley, No.1188 Lianhang Road, Minhang District, Shanghai, China.

URL: bowei-biotech.com

Contact: +8615221067868