TECHNICAL MANUAL

E2 (Estradiol) ELISA Kit

- **SKU CODE:** FSES0093
- **SIZE:** 48T/96T
- **DETECTION PRINCIPLE:** Competitive
- **RUO:** Research-Use-Only

Revised: 11/2025

E2 (Estradiol) ELISA Kit

Please read entire manual carefully before starting experiment. DO NOT mix reagents and use reagents from different kits or batches to prevent assay failure.

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1. Key Features

Detection Method:

Competitive (Antigen Coated)

Detection limit:

Muscle, Milk, Feed---5 ppb.

Cross-reactivity:

Estradiol ---100%; Estriol, Estrone, Ethinylestradiol, Diethylstilbestrol, Hexestrol,

Dienestrol --- < 1%

Sample recovery rate:

90%±30%.

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this ELISA Kit and/or components as described in section 4. Date of expiration is on the ELISA Box label.

3. Product Description

The Assay Genie Estradiol (E2) ELISA Kit is a highly sensitive assay for the quantitative measurement of a E2 in the following samples: muscle, milk, feed, etc.

Based on the competitive ELISA principle, the assay relies on the competition between free E2 in the sample and a pre-coated E2 -conjugated antigen immobilized on the microtiter plate for binding to a specific anti- E2 antibody. Following this competitive binding step, Horseradish Peroxidase (HRP) conjugate is introduced to form an enzyme-linked complex, which catalyzes a colorimetric reaction upon addition of the substrate reagent. The resulting optical density (OD) is measured spectrophotometrically and exhibits an inverse relationship with E2 concentration—higher E2 levels yield lower OD values. Quantification is achieved by comparing sample OD readings against a standard calibration curve provided in the kit.

Each component, including the antibody working solution, standards, and supplementary reagents, is optimized to ensure reproducibility and accuracy across a wide dynamic range.

4. Kit Contents

No	Component Name	Specifications	Storage
1	ELISA Microtiter plate	96 wells	Store the kit at 2-8°C. Do not freeze any test kit components.
2	Standard Liquid	1.5 mL each (ppb=ng/mL=ng/g) (0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb)	
3	HRP Conjugate	12 mL	
	Antibody Working Solution	7 mL	
4	Substrate Reagent A	7 mL	
5	Substrate Reagent B	7 mL	
7	Stop Solution	7 mL	
8	20x Concentrated Wash Buffer	20 mL	
9	Sample Diluent	50 mL	
10	Plate Sealer	3 pieces	
11	Sealed Bag	1 piece	
12	Technical Manual	1 copy	

Additional materials required:

1. 37°C incubator.

2. Plate Reader with 450nm filter.

3. Precision pipettes and disposable pipette tips.

4. Distilled water.

5. Disposable tubes for sample dilution.

6. Absorbent paper.

7. Acetonitrile, Acetone, NaOH, H3PO4 (≥85.0%), Trichloromethane

5. Precautions

1. This kit is ideal for research purposes only and not for diagnostics or therapeutic uses.

2. To identify the concentration of your target, a pilot experiment using standards and a small number of samples is recommended.

3. Store all components as listed in this manual. Do not use the ELISA Kit after its expiration date

4. Ensure unopened and unused plate is kept dry to avoid contamination.

5. Before using the kit, centrifuge tubes to spin down standard & antibodies.

6. Substrate Reagent should be abandoned if it turns blue color. When OD value of standard (concentration: 0) < 0.8 unit (A450nm < 0.8), it indicates the reagent may be deteriorated.

7. Wash steps are critical for the success of the assay, deviations from wash steps may cause false positives and result in a high background.

8. Duplicate wells are recommended for both standard and sample testing.

9. Do not let the microplate wells dry during assay.

10. Do not reuse tips and tubes to avoid cross contamination.

11. Avoid using the reagents from different batches together.

6. Experimental Preparation

The procedures outlined in this document are provided as general recommendations for sample preparation in ELISA assays. Due to the variability of biological samples and specific assay requirements, users are advised to optimize protocols based on their own experimental conditions.

***Note**: For information regarding validation data in specific samples, please contact our Technical Support Team at techsupport@assaygenie.com.*

Bring all reagents and samples to room temperature before use. Open the microplate reader in advance, preheat the instrument, and set the testing parameters.

A. Sample pretreatment Precautions

Experimental apparatus should be clean, and the pipette should be disposable to avoid cross-contamination during the experiment.

B. Solution preparation:

***Note:** Please prepare solution according to the number of samples. Don't use up all components in the kit at once.*

Solution 1: 0.2 M NaOH Solution

Dissolve 0.8 g of NaOH to 100 mL with deionized water. Mix fully.

Solution 2: 6 M H3PO4 Solution

Dilute 40 mL of H3PO4 to 100 mL with deionized water. Mix fully.

Solution 3: Wash Buffer

Dilute the 20xConcentrated Wash Buffer with deionized water.

(20xConcentrated Wash Buffer (V): Deionized water (V) =1:19).

C. Sample pretreatment procedure

1. Pretreatment of muscle (animal), feed, milk sample:

(1) Remove fat from sample, homogenize the sample with homogenizer.

(2) **Milk:** weigh 1±0.05 g of sample into the 50 mL centrifuge tube. Add 10 mL of deionized water, Vortex for 10 min. Weigh 1±0.05 g of sample solution into the 50 mL centrifuge tube. **Other:** weigh 1±0.05 g of homogenate fresh sample into the 50 mL centrifuge tube.

(3) Add 5 mL of **Acetonitrile** and 3 mL of **Acetone**, Vortex for 1 min.

(4) Centrifuge at 4000 r/min for 5 min at room temperature.

(5) Take 2 mL of supernatant into another centrifuge tube, dry at 50-60℃ with nitrogen evaporators or water bath. (Please do it in a ventilated environment.)

(6) Add 0.5 mL of **Trichloromethane** to dissolve the remaining dry material, then add 2 mL of **2M NaOH Solution** (Solution 1), vortex for 1 min.

(5) Centrifuge at 4000 r/min for 5 min at room temperature.

(6) Take 1 mL of supernatant into another centrifuge tube, add 150 µL of **6 M H3PO4 Solution** (Solution 2).

(7) Add 3 mL of **Acetonitrile** and 2 mL of **Acetone**, Vortex for 1 min. Centrifuge at 4000 r/min for 5 min at room temperature.

(8) Take 3 mL of supernatant into another centrifuge tube, dry at 50-60℃ with nitrogen evaporators or water bath.

(9) Add 0.5 mL of **Sample Diluent** to dissolve the remaining dry material, vortex for 1 min.

(10)Take 50 µL of supernatant for analysis.

Note: *Sample dilution factor: 8, detection limit: 6 ppb*

7. Assay Procedure

1. **Plate Setup:** Designate standard wells, test sample wells, and control (blank) wells on the pre-coated plate, and record their positions. It is recommended to measure each standard and sample in duplicate. ***Note:*** *Determine the optimal dilution ratio for samples through preliminary experiments or by following technical support recommendations*.
2. **Standard, Samples & Antibody Working Solution:** Add 50 µL of Standard or Sample per well, then add 50 µL of Antibody Working Solution. Cover the plate and incubate at 25°C for 30 minutes into each well**.** Incubate for 30 min at 25°C protected from light.
3. **Washing:** Uncover the sealer carefully, remove the liquid of each well. Immediately add 300 µL of Wash Buffer (Solution 3). Repeat wash procedure for 4 times, 30 s intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **HRP Conjugate:** add 100 µL of HRP Conjugate to each well, cover the plate sealer, oscillate for 10 s gently to mix thoroughly, incubate at 25°C for 30 min in shading light.
5. **Washing:** Repeat wash as instructed in step 3.
6. **Substrate Reagent:** Add 50 µL of Substrate Reagent A to each well, and then add 50 µL of Substrate Reagent B (1:1 ratio according to volume). Incubate 25°C for 15-20 min at in shading light. ***Note:*** *the reaction time can be shortened or extended according to the actual color change, but not more than 30 min. Preheat the Microplate Reader for about 15 min before OD measurement*.
7. **Stop Solution:** Add 50 µL of Stop Solution to each well and mix thoroughly. The colour will change to yellow immediately.
8. **OD Measurement:** Read the optical density (OD) at 450 nm in a microplate reader immediately after adding the Stop Solution.

8. Data Analysis

Absorbance% =A/A0×100%

A: Average absorbance of standard solution or sample

A0: Average absorbance of 0 ppb Standard solution

Calculate the mean OD450 value of the duplicate readings for each standard, control, and sample.

Create a four-parameter logistic curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis. (Remove the OD450 blank during plotting.) Alternatively, you can use curve fitting software. Excel software is not recommended.

Calculate the sample concentration by substituting OD450 value into the standard curve. Diluted samples should be multiplied by the relevant dilution ratio.

Note: *If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.*

9. ELISA Troubleshooting

Problem	Possible Causes	Solutions
Standard curve without signal	Incorrect reagent order; Mixed components from different kits; Missing reagents.	Ensure correct reagent order and use components from the same kit. Verify all reagents are added.
Overflow OD	Mixed components from different kits; Over-concentrated working solution	Use correct components and prepare solutions at recommended concentrations.
Poor standard curve	Incorrect curve fitting model.	Try alternative curve fitting models.
Samples without signal	Sample concentration too low; Incompatible buffer; Incorrect preparation; Sample degradation or excessive freeze-thaw.	Reduce dilution or concentrate sample. Check buffer compatibility and follow proper preparation and storage.
High CV%	Precipitate formation; Unclean plate; Foaming; Uneven washing; Incomplete reagent mixing; Pipetting inconsistency.	Dilute samples if needed, avoid foaming, ensure uniform washing, mix reagents thoroughly, and use calibrated pipettes.
Low standard signal	Improperly reconstituted standards; Degraded standards; Incorrect pipetting; Expired kit; Improper storage; Over-dried wells.	Reconstitute standards properly, use fresh kits, follow storage recommendations, and prevent wells from drying.
Slow colour development	TMB not equilibrated; Incorrect microplate reader wavelength; Over-washing.	Pre-warm TMB (30 min at 37°C), confirm correct wavelength (450 nm), and follow recommended washing times.
High background	Insufficient washing; Contaminated wash buffer; Excess detection reagents; Delayed reading; TMB exposed to light.	Wash adequately, prepare fresh wash buffer, use correct reagent amounts, read results promptly, and incubate TMB in the dark.

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.