Customer service: service@abbkine.com
Technical support: support@abbkine.com
Website: https://www.abbkine.com

EliKine™ Estradiol ELISA Kit

Cat #: KTE0003 Size: 48 T/96 T

	Estradiol ELISA Kit		
REF	**Cat #:** KTE0003	LOT	**Lot #:** Refer to product label
	Detection range: 50 pg/mL-1,200 pg/mL		**Sensitivity:** 50 pg/mL
	Precision: Intra-assay Precision: The CV (%) < 15%. Inter-assay Precision :The CV (%) < 15%		**Recovery:** The recovery ranged from 85% to 115% with an overall mean recovery of 100%.
	Specificity: EliKine™ Estradiol ELISA Kit has high sensitivity and excellent specificity for detection of Estradiol. No significant cross-reactivity or interference between Estradiol and analogues was observed.		
	Applicable samples: Serum, Plasma		
	Storage: The unopened kit should be stored at 4℃ for 12 months, protected from light.		

Assay Principle

Estradiol (E2) is a steroid, an estrogen, and the primary female sex hormone. It is named for and is important in the regulation of the estrous and menstrual female reproductive cycles. Estradiol is essential for the development and maintenance of female reproductive tissues such as the breasts, uterus, and vagina during puberty, adulthood, and pregnancy, but it also has important effects in many other tissues, including bone, fat, skin, liver, and the brain. While estrogen levels in men are lower compared to those in women, estrogens have essential functions in men, as well. It is found in most vertebrates and crustaceans, insects, fish, and other animal species. EliKine™ Estradiol ELISA Kit employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided has been pre-coated with a specific antibody for Estradiol. Standards or samples are added to the microtiter plate wells with Horseradish Peroxidase (HRP) conjugated Estradiol. The competitive inhibition reaction is launched between with HRP labeled Estradiol and unlabeled Estradiol with the antibody. A substrate solution is added to the wells and the color develops in opposite to the amount of Estradiol in the sample. The color development is stopped and the intensity of the color is measured.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Estradiol Standard	0.25 mL×6	0.5 mL×6	4℃
HRP Conjugated Estradiol	3 mL	6 mL	4℃
Assay Buffer	3 mL	6 mL	4℃
HRP Substrate A	3.5 mL	7 mL	4℃, protected from light
HRP Substrate B	3.5 mL	7 mL	4℃, protected from light

Stop Solution	3.5 mL	7 mL	4℃
Wash Buffer (20×)	7.5 mL	15 mL	4℃
Estradiol Microplate	48 wells	96 wells	4℃
Plate Covers	1	2	RT

Note: Std_1: 0 pg/mL; Std_2: 50 pg/mL; Std_3: 120 pg/mL; Std_4: 250 pg/mL; Std_5: 500 pg/mL; Std_6; 1,200 pg/mL.

Materials Required but Not Supplied

- Microplate reader capable of measuring absorbance at 450 nm
- Multi channel pipette or automated microplate washer
- Incubator, refrigerated centrifuge
- Precision pipettes, disposable pipette tips
- Deionized water

Reagent Preparation

Note: Bring all reagents equilibrate to room temperature before use. Follow the Microplate reader manual for set-up and preheat it for 15 min before OD measurement.

1×Wash Buffer: Wash Buffer(20×) dilute with deionized water 1:20 to obtain the 1×Wash Buffer. Store at 4℃. If crystals have formed in the Wash Buffer concentrates, warm them gently until they completely dissolved. Store at room temperature. Please note that 1×Wash Buffer is stable for 30 days.

Working Solution: Take Assay Buffer according to the volume of HRP Conjugated Estradiol 1/10, and mix with HRP Conjugated Estradiol to obtain Working Solution, before use. For example: Take 3 mL of HRP Conjugated Estradiol, add 0.3 mL of Assay Buffer and mix to get the Working Solution.

Sample Preparation

1. Serum: Use a serum separator tube and allow samples to clot for 30 min at room temperature before centrifugation for 15 min at 1,000 g. Remove serum and assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.
2. Plasma: Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 min at 1,000 g within 30 min of collection. Assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.

Note: Do not use grossly hemolyzed or lipemic specimens. If samples are to be used within 24 hours, they may be stored at 2 to 8℃. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Assay Procedure

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
2. Add 50 µL Standard or Sample per well. It is recommended that all Standards and Samples be added in duplicate to the microplate. Set a Blank well without any solution.
3. Add 50 µL Working Solution to each well (not to Blank well), Mix well, cover with the Plate Cover provided and then incubate for 1 h at 37℃.
4. Remove liquid in each well and wash, repeating the process for a total of three washes. Wash by filling each well with 1×Wash Buffer (250 µL) using a Multi channel pipette or automated microplate washer, and let it stand for 10 s, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining 1×Wash Buffer by invert the plate and blot it against clean paper towels.
5. Add 50 µL HRP Substrate A and 50 µL HRP Substrate B to each well, mix well and cover with the Plate Cover provided. Incubate for 15 min at 37℃. Keeping the plate away from drafts and other temperature fluctuations in the dark.
6. Add 50 µL Stop Solution to each well. Stop Solution should be added to the plate in the same order as HRP Substrate. The

color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

7. Determine the optical density of each well within 30 min, using a microplate reader set to 450 nm.

Data Analysis

1. Average the duplicate readings for each standard and sample.

2. Drawing of standard curve: With the standard solution concentration as the y-axis and the mean absorbance for each standard as the x-axis, draw the standard curve. A computer software can be used to create a standard curve.

Typical Data

Typical standard curve（$R^2 \geqslant 0.99$）

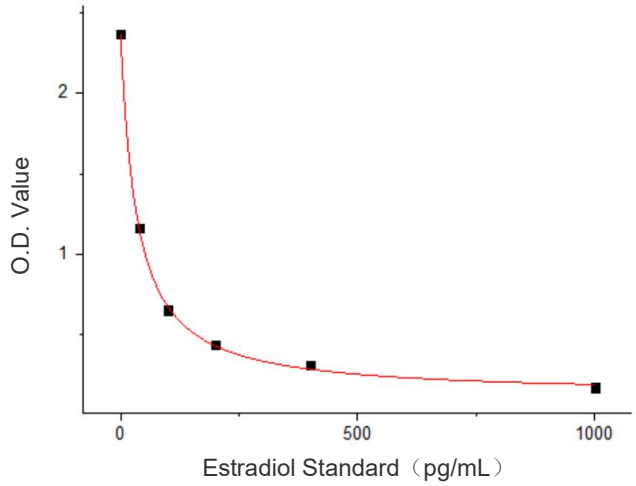

Figure. Standard Curve of Estradiol in 96-well plate assay, data provided for demonstration purposes only. A new standard Curve must be generated for each assay.

Precautions

1. Do not mix or substitute reagents with those from other lots or sources.

2. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

3. To ensure accurate results, proper adhesion of Plate Covers during incubation steps is necessary.

4. Stop Solution has certain Corrosive. Please take protective measures when operating.

Recommended Products

Catalog No.	Product Name
KTE0001	EliKine™ Testosterone ELISA Kit
KTE0002	EliKine™ Progesterone ELISA Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.