Signosis
Innovative Plate Assay Solutions

Testosterone ELISA Kit (Colorimetric)

Catalog Number EA-7072 **(For Research Use Only)**

Introduction

Testosterone is a key hormone primarily involved in the development and maintenance of male characteristics, though it is important in both men and women. It plays a vital role in regulating muscle mass, bone density, mood, and sex drive. Produced mainly by the testes in men and the ovaries in women, testosterone levels naturally fluctuate throughout life, peaking during adolescence and gradually declining with age. Because of its significant influence on physical health and well-being, researching testosterone is essential for understanding conditions like hormonal imbalances, infertility, and age-related changes, as well as for developing effective treatments.

Principle of the assay

The 96-well clear plate is coated with antibodies against the testosterone. When the sample, such as cell culture supernatant, cell lysate, tissue homogenate, serum, or plasma, is incubated in the hormone ELISA plate, the hormone binds to its corresponding capture antibody. After washing away the rest of the sample, the immobilized hormone is detected with a biotinylated detection antibody. The target hormone is thus sandwiched between its corresponding capture and detection antibodies. After incubating and washing away the unbound detection antibodies, a streptavidin-HRP conjugate is added to the plate and binds to the immobilized biotin-labeled detection antibodies. After incubating and washing away the unbound streptavidin-HRP, an HRP substrate, TMB, is added to the hormone sample which reacts with the HRP in the wells to form a blue color. The development of the blue color is then terminated with the addition of Stop Solution, which changes the blue color to yellow. The hormone levels in the samples are then determined by measuring the absorbance of the plate spectrophotometrically at 450 nm.

Diagram of Steroid Hormone ELISA

Materials provided with the kit

Component	Qty	Store at
96-Well Strip Plate coated with antibodies against Testosterone	1	4°C
Biotin-labeled antibody mixture against Testosterone	200 µL	-20°C
Streptavidin-HRP conjugate	50 µL	4°C
1x Diluent Buffer	40 mL	4°C
5x Assay Wash Buffer	40 mL	4°C
Substrate	10 mL	4°C
Stop Solution	5 mL	4°C

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  - 40 ml 5x Assay wash buffer
  - 160 ml ddH2O
- Dilute biotin-labeled antibody 50 times with 1x Diluent buffer.
- Dilute streptavidin-HRP 200 times with 1x Diluent buffer.

Sample preparation before starting experiment

- For **cell culture medium samples**, add 100µl directly to the well.
- For **cell lysate samples**, use cell lysis buffer (Catalog# EA-0001). Follow protocol in Cell Lysate Buffer User Manual.
- For **serum or plasma samples**, we recommend a 1:10 dilution with 1x Diluent buffer, for example, add 80µl sample in 720µl 1x Diluent buffer. When serum-containing conditional media is required, be sure to use serum as control.

Recommendation

- The product intends to be used for the comparison of different samples. The differences in hormone levels among the samples can be easily identified and measured.
- If you would like to quantitatively measure the steroid hormones in the samples, please order the hormone standards separately, which can be found on our website.

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Assay procedure

1. Take the desired number of well strips from the plate. Make sure the rest of strips are well sealed
2. Standard curve:
If a standard curve is desired, 4-5 strips may be used to make the standard curve.
3. Sample assay:
Apply each sample in one strip, 100µl per well and incubate for 1-2 hour at room temperature with gentle shaking.
4. Aspirate each well and wash by adding 200µl of 1x Assay wash buffer. Repeat the process three times for a total of three washes. Completely remove liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.
5. Add 100µl of diluted (1:50 in Diluent buffer) biotin-labeled antibody mixture to each well and incubate for 1 hour at room temperature with gentle shaking.
6. Repeat the aspiration/wash as in step 4.
7. Add 100µl of diluted (1:200 in Diluent buffer) streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
8. Repeat the aspiration/wash as in step 4.
9. Add 100µl substrate to each well and incubate for 10-30 minutes.

Note: Substrate incubation time may vary due to different antibodies reactivity. Stronger signals could be stopped early after 5-10 minutes. Weaker signals can be incubated longer for 30-60 minutes. **Always stop the reaction of the samples from the same row at the same time.**

10. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
11. Measure the absorbance of the plate at 450 nm with a microplate reader.