Signosis
Innovative Plate Assay Solutions

Steroid Hormone ELISA Combo Kit (Colorimetric)

Catalog Number EA-7070 **(For Research Use Only)**

Introduction

Signosis' Steroid Hormone ELISA Combo Kit allows researchers to analyze four critical steroid hormones – cortisol, testosterone, estradiol (E2), and progesterone – in a single assay. This multiplexed format offers a convenient solution for measuring multiple hormones with one 96-well plate. The array is designed for high sensitivity and specificity, utilizing an ELISA-based platform optimized for serum, cell and tissue lysates, or other biological fluids. This array is ideal for applications in endocrinology, stress research, reproductive biology, and clinical diagnostics. Whether you're studying hormone regulation, disease progression, or therapeutic response, the Steroid Hormone ELISA Combo Kit provides a powerful tool to accelerate your research.

Principle of the assay

The 96-well clear plate is coated with 4 different hormone antibodies, with 2 rows (24 wells) dedicated to each hormone target. This assay uses the competitive ELISA format, which is ideal for small-molecule biomarkers such as steroid hormones. Plates are pre-coated with steroid-BSA conjugates that compete with free markers in samples for binding to specific anti-steroid antibodies. The sample is added with the anti-steroid antibody to the plate. After washing away unbound material, HRP reagent binds to the anti-steroid antibodies (conjugated to biotin) in the plate. TMB substrate generates a blue color proportional to bound antibody, turning yellow upon acidification. Absorbance at 450 nm is inversely proportional to steroid concentration in the sample—higher free steroid levels block the antibodies from binding to the plate.

Materials provided with the kit

Component	Qty	Store at
96-Well Strip Plate coated with steroid-BSA conjugates	1	4°C
Biotin-labeled antibodies for 4 Steroid Hormones	4 tubes	-80°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 cortisol antibody 1:2000 with 1x Diluent buffer. (Make 1.3mL mix for 24 wells)
- Dilute biotin-labeled testosterone antibody 1:50 with 1x Diluent buffer. (Make 1.3mL mix for 24 wells)
- Dilute biotin-labeled estradiol antibody 1:500 with 1x Diluent buffer. (Make 1.3mL mix for 24 wells)
- Dilute biotin-labeled progesterone antibody 1:100 with 1x Diluent buffer. (Make 1.3mL mix for 24 wells)
- Dilute streptavidin-HRP 1:200 with 1x Diluent buffer.

Sample preparation before starting experiment

- For **cell culture medium samples**, add 50µl directly to the well. Samples can be diluted with 1x Diluent Buffer 1:2 or 1:4.
- For **cell lysate samples**, use lysis buffer to lyse cells and collect lysate. Lysate samples can be diluted with 1x Diluent Buffer 1:2 or 1:4.
- For **serum or plasma samples**, add 50µl directly to the well. Samples can be diluted with 1x Diluent Buffer 1:2 or 1:4.

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 individual steroid hormone kit and hormone standards separately, which can be found on our website.

Assay procedure

1. Add 50µL of sample to each well in the plate.
2. Add 50µL of corresponding diluted antibody mixture to each well with sample and mix. Incubate for 1 hour at room temperature with gentle shaking.
3. 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.
4. Add 100µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
5. Repeat the aspiration/wash as in step 3.
6. Add 100µl substrate to each well and incubate for 15-30 minutes.
7. When desired color intensity is obtained in the plate wells, add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
8. Immediately measure the absorbance of the plate at 450 nm with a microplate reader.

Steroid Hormone ELISA Array Diagram

	1	2	3	4	5	6	7	8	9	10	11	12
A	Cortisol (12 wells)											
B	Cortisol (12 wells)											
C	Testosterone (12 wells)											
D	Testosterone (12 wells)											
E	Estradiol (E2) (12-wells)											
F	Estradiol (E2) (12-wells)											
G	Progesterone (12 wells)											
H	Progesterone (12 wells)											