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. 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 mixture of biotinylated detection antibodies. 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 Array

Materials provided with the kit

Component	Qty	Store at
96-Well Strip Plate coated with antibodies against 4 Steroid Hormones	1	4°C
Biotin-labeled antibody mixture against 4 Steroid Hormones	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 mixture 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.

Steroid Hormone ELISA Array Diagram

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