MULTI SCIENCES

Cortisol Competitive ELISA Kit

【Catalog No】EK8100

【SIZE】48T/96T

【INTENDED USE】For the quantitative determination of cortisol concentration in cell culture supernates, serum and plasma.

【INTRODUCTION】

Cortisol, also known as hydrocortisone or compound F, is a steroid hormone, in the glucocorticoid class of hormones. It is produced in humans by the zona fasciculata of the adrenal cortex within the adrenal gland. It is released in response to stress and low blood-glucose concentration, therefore, cortisol can be used as a biomarker of stress. Production of cortisol follows an ACTH-dependent circadian rhythm, with peak levels in the morning and decreasing levels throughout the day. Cortisol can be measured in many matrices including blood, feces, urine, and saliva. Most serum cortisol (90 - 95 %) is bound to proteins including corticosteroid binding globulin and serum albumin.

Cortisol is involved primarily in metabolic and immunological actions. In the metabolic aspect, it promotes gluconeogenesis, liver glycogen deposition, and the reduction of glucose utilization. Immunologically, Cortisol functions as an important anti-inflammatory, and plays a role in hypersensitivity, immunosuppression, and disease resistance. Abnormal Cortisol levels are being tested for correlation with a variety of different conditions, these include: prostate cancer, depression, and schizophrenia. It is already known that an excess of Cortisol in all bodily tissues is the cause of Cushing's Syndrome.

【PRINCIPLE OF THE ASSAY】

This assay employs a solid-phase competitive Enzyme-Linked Immunosorbent Assay (ELISA) technique. Rabbit anti-mouse IgG polyclonal antibody has been pre-coated onto a microplate. Monoclonal antibody specific for cortisol is added into the wells, and is bound by the immobilized antibody following incubation. After washing, HRP-labeled cortisol and purified cortisol/sample is added to compete for limited sites on the monoclonal antibody. After washing away any unbound substances, substrate solution is added to the wells and color develops in inverse proportion to the amount of cortisol. The color development is stopped and the intensity of the color is measured. The assay is validated with serum samples from human, mouse and rat, but is expected to measure cortisol in samples from other species.

【MATERIALS PROVIDED】

PART	PART #	EK8100-48	EK8100-96
Microplate Wells	EK801050P	48T	96T
Cortisol Antibody	EK8100A	1 vial	1 vial
Cortisol Standard	EK8100S1	1 vial	1 vial
Cortisol Conjugate	EK8100S2	1 vial	1 vial
Standard Diluent	E0260	5ml	5ml
Dissociation Reagent	E0306	10ml	10ml
10×Assay Buffer	E0310	10ml	10ml
Substrate TMB	E0230	6ml	11ml
Stop Solution	E0300	11ml	11ml
20×Washing Buffer	E0281	50ml	50ml
Adhesive Film	E0200	6	6

Note: Components from reagent kits of different batch numbers must not be used interchangeably.

OTHER SUPPLIES REQUIRED

1) Microplate reader capable of measuring absorbance at 450 nm, with correction wavelength set at 570 nm or 630 nm.

2) Pipettes and pipette tips.

3) 50 µl to 300 µl adjustable multichannel micropipette with disposable tips.

4) Multichannel micropipette reservoir.

5) Beakers, flasks, cylinders necessary for preparation of reagents.

6) Deionized or distilled water.

7) Polypropylene test tubes for dilution.

【STORAGE】

Store at 2-8℃; refer to the kit label for expiration date.

For opened kits:

Pre-coated microplate: Can be stored at 2-8℃ for approximately 1 month (return unused strips to the aluminum foil bag and reseal).

Standard: Can be stored at -20℃ for approximately 1 month (discard after single-use reconstitution).

Other components: Can be stored at 2-8℃ for approximately 1 month.

【SAMPLE COLLECTION AND STORAGE】

1) **Cell Culture Supernates** – Remove particulates by centrifugation and assay freshly prepared samples immediately or aliquot and store samples at ≤ -20℃ for later use. Avoid repeated freeze-thaw cycles.

2) **Serum** – Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 10 minutes at 1,000 × g. Remove serum and assay freshly prepared samples immediately or aliquot and store samples at ≤ -20℃ for later use. Avoid repeated freeze-thaw cycles.

3) **Plasma** – Collect plasma using EDTA, citrate or heparin as anticoagulant. Centrifuge at 1,000 × g within 30 minutes of collection. Assay freshly prepared samples immediately or aliquot and store samples at ≤ -20℃ for later use. Avoid repeated freeze-thaw cycles.

4) Other biological samples might be suitable for use in the assay. Cell culture supernates, serum and plasma were tested with this assay. Dilution with Assay Buffer may be needed.

Note: Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

If samples are to be run within 24 hours, they may be stored at 2 to 8℃. For longer storage, aliquot samples and store frozen at -20℃. Avoid repeated freeze-thaw cycles.

【SAMPLE PREPARATION】

For serum samples, pretreatment should be performance to remove potentially interfering proteins and cortisol-binding protein.

1. Add 100 µl serum and 100 µl Dissociation Reagent to a microcentrifuge tube. Mix well.
2. Incubate for 15 minutes at room temperature. Centrifuge at ≥ 12,000 × g for 4 minutes.
3. Pipet 50 µl supernate carefully, and transfer to a new microcentrifuge tube, add 950 µl Assay Buffer (1×), mix well.

【REAGENT PREPARATION】

Bring all reagents and samples to room temperature before use.

If crystals form in the Buffer Concentrates, warm and gently stir them until completely dissolved.

Washing Buffer (1×)

Pour entire contents (50 ml) of the **Washing Buffer (20×)** into a clean 1,000 ml graduated cylinder. Bring to final volume of 1,000 ml with pure or deionized water.

Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2 to 25℃. Washing Buffer (1×) is stable for 30 days.

Assay Buffer (1×)

Pour the entire contents (5 ml) of the **Assay Buffer (10×)** into a clean 100 ml graduated cylinder. Bring to final volume of 50 ml with distilled water. Mix gently to avoid foaming.

Store at 2 to 8℃. Assay Buffer (1×) is stable for 30 days.

Cortisol Antibody

Briefly spin the vial before opening. Reconstitute by addition of distilled water. Reconstitution volume is stated on the label of the vial. Allow the antibody to reconstitute for 15 minutes. Mix well prior to making dilutions.

Make a **1：50** dilution of the concentrated **Cortisol Antibody** solution with Assay Buffer (1×) in a clean plastic tube as needed.

The diluted Cortisol Antibody should be used within 30 minutes after dilution.

Cortisol Conjugate

Thaw thoroughly at room temperature (25℃ ± 3℃) before use. Briefly spin the vial before opening.

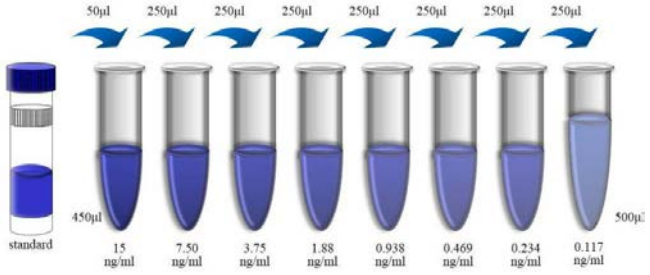
Make a **1：100** dilution of the concentrated **Cortisol Conjugate** solution with Assay Buffer (1×) in a clean plastic tube as needed.

The diluted Cortisol Conjugate should be used within 30 minutes after dilution.

Sample Dilution: If your samples have high **Cortisol Competitive** content, dilute serum/plasma samples with Assay Buffer (1×). For cell culture supernates, dilute with cell culture medium.

Cortisol Competitive standard:The concentration of provided Cortisol Standard is 150 ng/ml. The Cortisol Standard contains an organic solvent. Pipette the Standard up and down several times to wet the pipet tip before transfer to insure that volumes are dispensed accurately.Use polypropylene tubes.

- **For serum/plasma samples,** mixing concentrated cortisol standard (50 µl) with 450 µl of Standard Diluent creates the high standard (15 ng/ml). Pipette 250 µl of Standard Diluent into each tube. Use the high standard to produce a 1:1 dilution series (scheme below). Mix each tube thoroughly before the next transfer. Standard Diluent serves as the zero standard (0 pg/ml).
- **For cell culture supernates,** mixing concentrated cortisol standard (50 µl) with 450 µl of cell culture medium without serum creates the high standard (15 ng/ml). Pipette 250 µl of cell culture medium without serum into each tube. Use the high standard to produce a 1:1 dilution series. Mix each tube thoroughly before the next transfer. Cell culture medium without serum serves as the zero standard (0 pg/ml).



【PLATE SETUP】

Each plate or set of strips must contain a minimum of two Blanks, two non-specific binding wells (NSB), two maximum binding wells (B0), and an eight point standard curve run in duplicate.

Note: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results. Each sample should be assayed at two dilutions and each dilution should be assayed in duplicate. For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format and pipetting summary are shown below. The user may vary the location and type of wells present as necessary for each particular experiment..

Component / Well	Standard Diluent	Antibody	Standard	Sample	Conjugate
Blank	-	-	-	-	-
NSB	100 µl	-	-	-	50 µl
B_0	100 µl	50 µl	-	-	50 µl
TA	-	-	-	-	5 µl (at develop step)
Standard	-	50 µl	100 µl	-	50 µl
Sample	-	50 µl	-	100 µl	50 µl

Blank: background absorbance.

NSB (Non-Specific Binding): non-immunological binding of the conjugate to the well.

TA (Total Activity): total enzymatic activity of the conjugate.

B0 (Maximum Binding): maximum amount of the conjugate that the antibody can bind in the absence of free analyte.

【ASSAY PROCEDURE】

Bring all reagents and samples to room temperature before use.

1) Prepare all reagents including microplate, samples, standards and working solution as described in the previous sections.

2) Remove excess microplate strips and return them to the foil pouch containing the desiccant pack, and reseal for further use.

3) Add 300 µl Washing Buffer (1×) per well, and allow it for about 30 seconds before aspiration. Soaking is highly recommended to obtain a good test performance. Empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Washing Buffer (1×). Use the microwell strips immediately after washing. Do not allow wells to dry.

4) Add 50 µl of diluted **Antibody** to each well except **Blank, NSB** and **TA** wells.

5) **Seal the plate with an adhesive film.** Incubate at room temperature (25℃±3℃)for 1.5 hours on a microplate shaker set at 300 rpm.

6) Aspirate each well and wash, repeating the process five times for a total six washes. Wash by filling each well with 300 µl Washing Buffer (1×). Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Washing Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

7) Add 100 µl of serial diluted Standard to Standard wells in duplicate. Add 100 µl of prepared samples to Sample wells. Add 100 µl of Standard Diluent to **NSB** and **B0** wells.

8) Add 50 µl of diluted Cortisol Conjugate to each well except Blank and TA wells. Ensure reagent addition in step 7 and 8 is uninterrupted and completed within 15 minutes.

9) **Seal the plate with an adhesive film.** Incubate at room temperature (25℃±3℃)for 2 hours on a microplate shaker set at 300 rpm.

10) Repeat aspiration/wash as in step 6.

11) Add 5 µl of diluted Cortisol Conjugate to **TA** well.

12) Add 100 µl of Substrate Solution to each well. Incubate for 5 - 30 minutes at room temperature(25℃±3℃). Protect from light.

13) Add 100 µl of Stop Solution to each well. The color will turn yellow. If the color in the well is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

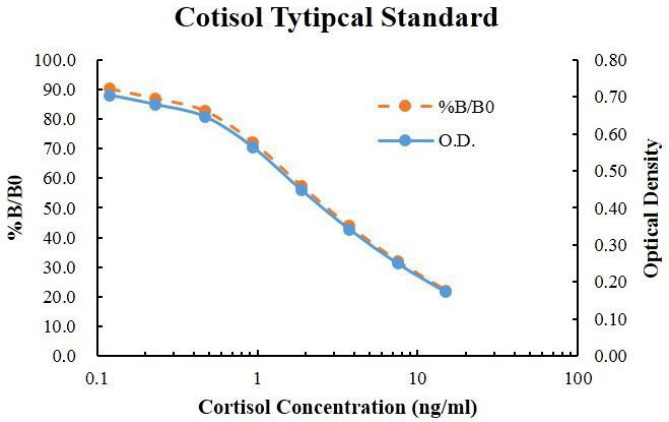
14) Measure the optical density value within 30 minutes by microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This

subtraction will correct for optical imperfections in the plate. Reading directly at 450 nm without correction may generate higher concentration than true value.

【TYPICAL DATA】

A standard curve must be run within each assay. The following standard curve is provided for demonstration only.

ng/ml	O.D.		Average	Corrected	%B/B0
NSB	0.021	0.022	0.022	-	-
B0	0.800	0.804	0.802	0.781	-
0.12	0.732	0.719	0.726	0.704	90.14
0.23	0.697	0.705	0.701	0.679	86.93
0.47	0.669	0.667	0.668	0.646	82.71
0.94	0.579	0.591	0.585	0.563	72.09
1.88	0.469	0.471	0.470	0.448	57.36
3.75	0.362	0.366	0.364	0.342	43.79
7.50	0.266	0.278	0.272	0.250	32.01
15.00	0.194	0.195	0.195	0.173	22.15



MULTI SCIENCES BIOTECH CO., LTD.

www.multisciences.net

E-mail: service@multisciences.net