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Product Information

Sheep Cortisol ELISA

Catalog Number **SE120115**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Cortisol (hydrocortisone, compound F) is the most potent glucocorticoid synthesized from cholesterol. Cortisol is found in the blood either as free cortisol or bound to corticosteroid-binding globulin (CBG). Cortisol production has an ACTH-dependent circadian rhythm with peak levels in the early morning and a nadir at night. The factors controlling this circadian rhythm are not completely defined. Serum levels are highest in the early morning and decrease throughout the day. In the metabolic aspect, cortisol 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.

The Sheep Cortisol ELISA is used for the quantitative measurement of cortisol in sheep serum or plasma. It is a solid phase competitive ELISA. The samples and cortisol enzyme conjugate are added to the wells coated with anti-cortisol monoclonal antibody. Cortisol in the sample competes with a cortisol enzyme conjugate for binding sites. Unbound cortisol and cortisol enzyme conjugate are washed off. Upon the addition of the substrate, the intensity of color is inversely proportional to the concentration of cortisol in the samples. A standard curve is prepared relating color intensity to the concentration of the cortisol. This kit can detect low level of cortisol in sheep serum or plasma (less than 0.1 ng/ mL).

Components

Materials Provided	96 tests
Microwell coated with Cortisol MAb	12 x 8 x 1
Cortisol Standard: 7 vials (ready to use)	0.7 mL
Enzyme Conjugate (20x)	1.2 mL
Assay Diluent	24 mL
TMB Substrate: 1 bottle (ready to use)	12 mL
Stop Solution: 1 bottle (ready to use)	12 mL
20x Wash concentrate: 1 bottle	25 mL

Reagents and Equipment Required but Not Provided.

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbent paper or paper towel
6. Graph paper

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2–8 ° C) for 5 days. If storage time exceeds 5 days, store frozen at (–20 °C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

Cortisol-enzyme Conjugate Solution

Dilute the ortisol enzyme conjugate 21-fold with assay diluent in a suitable container. For example, dilute 100 µL of conjugate with 2 mL of assay diluent buffer for 10 wells (A slight excess of solution is made).

20x Wash Buffer Concentrate

Prepare 1× Wash buffer by adding the contents of the bottle (25 mL, 20×) to 475 mL of distilled or deionized water. Store at room temperature.

Storage/Stability

Store the kit at 2–8 °C. Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit.

Procedure

Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

It is recommended that standards, control, and serum samples be run in duplicate.

Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

Prior to sssay, allow reagents to stand at room temperature. Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder.
2. Pipette 40 μL of cortisol standards, control, and sera.
3. Add 200 μL of Cortisol Enzyme Conjugate to all wells.
4. Incubate for 60 minutes at room temperature (18–26 °C) with shaking.
5. Remove liquid from all wells. Wash wells three times with 300 μL of 1x Wash buffer. Blot on absorbent paper towels.
6. Add 100 μL of TMB substrate to all wells.
7. Incubate for 15 minutes at room temperature (18–26 °C) with shaking.
8. Add 50 μL of Stop Solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 20 minutes after adding the stop solution.

Results

The standard curve is constructed as follows:

1. Check Cortisol standard value on each standard vial. This value might vary from lot to lot. Make sure the value is checked on every kit.
2. To construct the standard curve, plot the absorbance for cortisol standards (vertical axis) versus cortisol standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Typical Data for Standard Curve

Standard	Concentration (ng/ mL)	OD (450 nm)
1	0	2.62
2	1	2.37
3	5	1.65
4	10	1.24
5	20	0.83
6	40	0.59
7	80	0.33

Product profile:

Correlation with a Reference ELISA kit

A total of 86 sera were tested by Sheep Cortisol ELISA and a reference ELISA kit. Results were as follows:

Correlation	Slope	Intercept
0.95	0.96	0.5

Precision

Intra-Assay Precision was determined by assaying 16 replicates of each of three sera; low, normal, and high.

Serum	No. of Replicates	Mean ng/mL	Standard Deviation	Coefficient of Variation (%)
1	16	40.1	2.54	6.3
2	16	59.4	5.57	9.4
3	16	165.5	10.23	6.2

Inter-assay Precision was determined by assaying duplicates of three serum pools in 10 separate runs, using a standard curve constructed for each run.

Serum	No. of Runs	Mean ng/mL	Standard Deviation	Coefficient of Variation (%)
1	10	52	7.8	15
2	10	87	8.9	10.2
3	10	156	13.5	8.6

Sensitivity

This assay sensitivity was found to be 0.1 ng/mL. The sensitivity was determined by calculating the mean plus 2 SD of the standard zero point tested 20 times in the same run.

Specificity

Steroid	**Cross reactivity**
Cortisol	100%
Prednisolone	5.1%
11-Deoxycortisol	0.6%
Corticosterone	0.3%
11-Deoxycorticosterone	<0.1%
Progesterone	<0.1%
17-OH-Progesterone	<0.1%
Estradiol	<0.1%
Testosterone	<0.1%

References

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2 Stewart, P.M. et al., A rational approach for assessing the hypothalamo-pituitary-adrenal axis. Lancet, 5:1208-1210, 1988.

3 Watts, N.B., and Tindall, G.T., Rapid assessment of corticotropin reserve after pituitary surgery. JAMA, 259:708-711, 1988.

4 Tiertz, N.W., in: Text book of Clinical Chemistry, Saunders, 1968

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