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Product Information

Mouse/Rat Cortisol ELISA

Catalog Number **SE120082**
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. It has also been shown that plasma cortisol levels elevate in response to stress.

The Mouse/Rat Cortisol ELISA is intended for the quantitative measurement of cortisol in mouse/rat 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.

Components

Materials Provided	96 Tests
Microwells coated with Cortisol MAb	12 x 8 x 1
Cortisol Standard: 7 vials (ready to use)	0.5 mL
Enzyme Conjugate (20x)	0.7 mL
Assay Diluent,	12 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.

20× Enzyme Conjugate

Prepare 1× working solution by diluting 20-fold with assay diluent as needed (*e.g.*, 0.1 mL of the 20× Enzyme Conjugate in 1.9 mL of Assay Diluent is sufficient for 20 wells). The diluted conjugate has to be used the same day.

20x Wash Buffer Concentrate

Prepare 1x wash buffer by adding the contents of the bottle to 475 mL of distilled water. Store 1x Wash buffer 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. Do not expose test reagents to heat, sun, or strong light.

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

Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.

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

Prior to assay, 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 25 µL of Cortisol standards, control, and patient's sera.
3. Add 100 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).
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.

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