3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Cortisol ELISA

Catalog Number **SE120037**
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. 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. Abnormal cortisol levels are seen with a variety of different conditions: adrenal tumors, prostate cancer, depression, and schizophrenia. Elevated cortisol levels and lack of diurnal variation have been identified in patients with Cushing's disease

This kit 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 patient's sample competes with a cortisol enzyme conjugate for binding sites. Unbound cortisol and cortisol enzyme conjugate are washed off by washing buffer. 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.

The Cortisol ELISA Kit is intended for the quantitative measurement of cortisol in human serum or plasma.

Components

Materials Provided	96 Tests
Microwells coated with Cortisol MAb	12 × 8 × 1
Cortisol Standard: 7 vials (ready to use)	0.5 ml
Enzyme Conjugate (20×)	1.2 ml
TMB Substrate: 1 bottle (ready to use)	12 ml
Stop Solution: 1 bottle (ready to use)	12 ml
20× Wash concentrate: 1 bottle	25 ml
Assay Diluent	24 ml

Reagents and Equipment Required but Not Provided.

- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- ELISA reader capable of reading absorbance at 450 nm
- Absorbent paper or paper towel
- 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.

20× 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 (18–26 °C).

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.

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

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.

All reagents must be brought to room temperature (18–26 °C) before use and gently mix.

1. Place the desired number of coated strips into the holder.
2. Pipette 25 µl of cortisol standards, control and patient's sera into selected wells.
3. Add 200 µl of Enzyme Conjugate to all wells.
4. Thoroughly mix for 10 seconds.
5. Incubate for 60 minutes at room temperature (18–26 °C).
6. Remove liquid from all wells. Wash wells three times with 300 µl of 1× wash buffer. Blot on absorbent paper towels.
7. Add 100 µl of TMB substrate to all wells.
8. Incubate for 15 minutes at room temperature (18–26 °C).
9. Add 50 µl of Stop Solution to all wells. Shake the plate gently to mix the solution.
10. 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.

   Example of Standard Data

	OD 450 nm	Concentration (ng/ml)
Std 1	2.36	0
Std 2	1.76	20
Std 3	1.10	50
Std 4	0.65	100
Std 5	0.29	200
Std 6	0.13	400
Std 7	0.08	800

3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Expected Values
It is recommended each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values for cortisol may be used as initial guideline ranges only:

Classification	ng/ml
8:00 am – 10:00 am	50–230
4:00 pm	30–150

Limitations of the Test
1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings, and other diagnostic procedures.
2. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

References

1. Chernow, B. et al., Hormonal responses to graded surgical stress. Arch. Intern. Med., **147**, 1273-1278 (1987).
2. Crapo, L., Cushing's syndrome: A review of diagnostic tests. Metabolism, **28**, 955-977 (1979).
3. Lee, P.D.K. et al., Virilizing adrenocortical tumors in childhood. Eight cases and a review of the literature. Pediatrics, **76**, 437-444 (1985).
4. Leisti, S. et al., The diagnosis and staging of hypocortisolism in progressing autoimmune adrenalitis. Pediatr. Res., **17**, 861-867 (1983).
5. Stewart, P.M. et al., A rational approach for assessing the hypothalamo-pituitary-adrenal axis. Lancet, **5**, 1208-1210 (1988).
6. Watts, N.B., and Tindall, G.T:, Rapid assessment of corticotropin reserve after pituitary surgery. JAMA, **259**, 708-711 (1988).
7. Schlaghecke, R. et al., The effect of long-term glucocorticoid on pituitary-adrenal responses to exogenous corticotropin-releasing hormone. New Engl. J. Med., **326**, 226-230 (1992).
8. Tiertz, N.W., in Text book of Clinical Chemistry, Saunders, 1968.

SG,CH,MAM 10/14-1