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## Product Information

### Anti-Cortisol

produced in rabbit, whole antiserum

Catalog Number **C8409**

### Product Description

The antiserum is produced in rabbit using as the immunogen cortisol-21-hemisuccinyl-thyroglobulin. The antibody is provided as a pre-diluted antiserum that has been lyophilized.

### Reagent

Supplied as lyophilized powder. Each vial contains no more than 20 mg Polyvinylpyrrolidone (PVP).

### Precautions and Disclaimer

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

### Reconstitution and Dilution

- **Stock Solution:** To one vial of lyophilized powder add 5.0 mL of 0.05 M Tris-HCl buffer, pH 8.0, containing 0.1 M NaCl, 0.1% gelatin and 15 mM sodium azide. Rotate vial gently until powder is dissolved.
- **Working Solution:** To obtain the number of tests indicated on the vial, further dilute the reconstituted antiserum 10-fold with the buffer used to prepare the stock solution.

### Storage

Prior to reconstitution, store at 2-8 °C.

After reconstitution:

- **Stock Solution:** Separate into aliquots and freeze. Repeated freezing and thawing is **not** recommended.
- **Working Solution:** Discard if unused within 12 hours.

### RIA SYSTEM

#### RIA Characterization

The antiserum is characterized utilizing the following dextran coated charcoal radioimmunoassay (RIA) protocol, where 0.5 mL of reconstituted and diluted antiserum has been found to bind at least 40-60% of 5 picograms of tritiated (<sup>3</sup>H) cortisol with a specific activity of approximately 100 Ci/mmole.

**Note:** It is recommended that the antiserum first be evaluated in the assay system described due to differences in systems and procedures.

### RIA Reagents

- (A) **Standards:** Prepare a stock standard solution of 1 µg/mL cortisol (hydrocortisone, Catalog Number H4001) in absolute ethanol. Dilute a portion of the stock solution with buffer (B) to a concentration of 250 pg/0.1 mL. This is further diluted in buffer (B) to obtain standard solutions at the following concentrations: 125, 63, 31 and 15 pg/0.1 mL.
- (B) **Dilution buffer:** 0.05 M Tris-HCl (Catalog Number T3253), pH 8.0, containing 0.1 M NaCl, 0.1% gelatin (Catalog Number G2500) and 15 mM sodium azide.
- (C) **Dextran coated charcoal suspension:** 0.5% activated charcoal untreated powder 250-350 mesh, 0.05% dextran approximate average molecular weight 70,000 (Catalog Number D1390) in buffer (B). It is important that the dextran be in solution before the addition of charcoal. The dextran coated charcoal suspension should be stirred and kept at 0 °C in ice-water for at least 30 minutes before and during use.

### RIA Protocol

1. Pipette 0.1 mL sample or standard (A) and 0.5 mL diluted antiserum into assay tubes.
2. Vortex the tubes.
3. Incubate for 30 minutes at room temperature.
4. Add 0.1 mL tritiated radioactive tracer diluted in dilution buffer (B).
5. Vortex the tubes.
6. Incubate for 1 hour at 37 °C.
7. Cool the tubes for 15 minutes at 4 °C.
8. Rapidly add 0.2 mL cold dextran coated charcoal suspension (C) to each tube.
9. Vortex the tubes.
10. Incubate for 10 minutes at 0°C in ice-water.
11. Centrifuge at 2000 x g for 15 minutes at 4 °C.
12. Remove 0.25 mL supernatant from each tube, add 3.0 scintillation cocktail to the supernatant and determine the amount of radioactivity present.

### RIA Specificity

Specificity of the antiserum is defined as the ratio of antigen concentration to cross-reactant concentration at 50% inhibition of maximum binding. The cross-reactivity data obtained in the described RIA system is as follows:

Cross-Reactant	%Cross-Reactivity
Cortisol	100
Androstenedione	< 0.5
Compound S	<15
Corticosterone	<20
Deoxycorticosterone	<10
Progesterone	<10
Testosterone	<5

### RIA Sensitivity

Sensitivity is defined as the 90% intercept of a B/B<sub>0</sub> standard curve. In the above system the sensitivity has been found to be 10 pg/tube.

### RIA Affinity Constant

The affinity constant (K<sub>a</sub>) is determined by a Scatchard plot using the described RIA system.

K<sub>a</sub> = 1-10 x 10<sup>9</sup> L/mole.

### Cortisol Levels

Normal plasma cortisol

(8 am) 12,800 ± 4,000 pg/0.1ml

Hyperadrenalism

(e.g., severe operation) 60,000 pg/0.1ml

Hypoadrenalism

(e.g., Addison's disease,  
pituitary insufficiency) <1000 pg/0.1ml

### References

1. Ruder, H., et al., *J. Clin. Endocr. Metab.*, **35**, 219 (1972).
2. Vecsei, P., In: *Methods of Hormone Radioimmunoassay* Jaffe, B., and Behrman, H., (Eds.), Academic Press, New York, p. 393 (1974).

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