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Product Information

Anti-Corticosterone antibody
Produced in rabbit, Whole Antiserum

Catalog Number **C8784**

Product Description

The antiserum is developed in rabbit using corticosterone-21-thyroglobulin as the immunogen. The product is provided as a pre-diluted antiserum that has been lyophilized. Each vial contains no more than 20 mg Polyvinylpyrrolidone (PVP).

Precautions and Disclaimer

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

1. Stock Solution: To one vial of lyophilized powder add 5.0 ml of the dilution buffer (0.05 M Tris-HCl buffer, pH 8.0, containing 0.1 M sodium chloride, 0.1% bovine serum albumin, and 0.1% sodium azide). Rotate vial gently until powder is dissolved.
2. 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/Stability

Prior to reconstitution store at 2–8 °C.
After reconstitution:

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

Procedure

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% of 15 picograms of tritiated (^{3}H) corticosterone with a specific activity of approximately 100 Ci/mmole.

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

RIA Reagents

- Dilution buffer:
  0.05 M Tris-HCl (Catalog Number T3253), pH 8.0, containing 0.1 M NaCl, 0.1% BSA (Catalog Number A7030), and 0.1% sodium azide.
- Standards:
  Prepare a stock standard solution of 1 μg/ml corticosterone (Catalog Number C2505) in absolute ethanol. Dilute a portion of the stock solution with Dilution buffer to a concentration of 2,000 pg/0.1 ml. This is further diluted in Dilution buffer to obtain standard solutions at the following concentrations: 1,000, 500, 250, 125, and 63 pg/ml.
- Dextran coated charcoal suspension:
  0.5% activated charcoal untreated powder 100-400 mesh, 0.5% dextran approximate average molecular weight 70,000 (Catalog Number D1390) in Dilution buffer. 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. In polypropylene test tubes, add 0.1 ml sample or standard (A) and 0.5 ml diluted antiserum.
2. Vortex the tubes.
3. Incubate for 30 minutes at room temperature.
4. Add 0.1 ml tritiated radioactive tracer diluted in Dilution buffer.
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 × *g* for 15 minutes at 4 °C.
12. Remove supernatant from each tube, add scintillation cocktail to the supernatant and determine the amount of radioactivity present.

Results

RIA Sensitivity

Sensitivity is defined as the 90% intercept of a B/B_0 standard curve. In the described system the sensitivity has been found to be 30 picograms/tube.

RIA Affinity Constant

The affinity constant (K_a) is determined by a Scatchard plot using the described RIA system.

$$K_a = 1–10 \times 10^9 \text{ L/mole.}$$

References

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