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ProductInformation

ANTI-ESTRONE
Developed in Rabbit
Whole Antiserum

Product Number **E 3135**

Product Description
The antiserum is developed in rabbit using estrone-6-thyroglobulin as the immunogen.

Reagents
The product is provided as a pre-diluted antiserum that has been lyophilized containing no more than 20 mg Polyvinylpyrrolidone (PVP).

Precautions and Disclaimer
Due to the PVP and sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Reconstitution
1. Stock Solution: To one vial of lyophilized powder add 5.0 ml of 0.05 M Tris-HCl buffer, pH 8.0, containing 0.1M sodium chloride, 0.1% gelatin and 15mM 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 freeze ib aliquots.
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Repeated freezing and thawing is **not** recommended. Discard working solution if unused within 12 hours.

Procedure
The antiserum is characterized utilizing the following dextran coated charcoal radioimmunoassay (RIA) protocol.

Reagents
A. Standards: Prepare a stock standard solution of 1 µg/ml estrone (Product No. E 9750) in absolute ethanol. Dilute a portion of the stock solution with buffer (B) to a concentration of 1000 pg/0.1 ml. This is further diluted in buffer (B) to obtain standard solutions at the following concentrations: 500, 250, 125, 63, 31 and 15 pg/0.1 ml.
B. Dilution buffer: 0.05 M Tris-HCl (Product No. T 3253), pH 8.0, containing 0.1 M NaCl, 0.1% Gelatin (Product No. G 2500), and 0.1% sodium azide.
C. Dextran coated charcoal suspension: 0.5% activated charcoal untreated powder 100-400 mesh (Product No. C 3345), 0.1% dextran approximate average molecular weight 70,000 (Product No. D 1390) 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. 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 (B).
5. Vortex the tubes.
6. Incubate for 1 hour at 37 °C or for 18-20 hours at 4 °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 supernatant from each tube,add scintillation cocktail to the supernatant and determine the amount of radioactivity present.

References

1. Kohen, F., et al., Steroid Immunoassay (Cameron, E.H.D., et al., eds., Alpha Omega Publ., Cardiff, Wales), p. 11 (1975).
2. Emment, Y., and Collin, W. P., Acta Endocrinol (copenh.)., **69**, 567 (1972).

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