

Product No. H-5140
Anti-17 α -Hydroxyprogesterone
Developed in Rabbit
Whole Antiserum

Lot 112H48251

The antiserum is developed in rabbit using 17 α -Hydroxyprogesterone-7 α -BSA as the immunogen. The product is provided as a lyophilized, pre-diluted antiserum.*

Reconstitution and Dilution

1. **Stock Solution:** To one vial of lyophilized powder add 5.0 ml of 0.05 M Tris-HCl buffer, pH 8.0, containing 0.1% gelatin 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

Prior to reconstitution store at 0-5°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.

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 10 picograms of tritiated (³H) 17 α -hydroxyprogesterone with a specific activity of approximately 40 Ci/mmol.

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 μ g/ml 17 α -hydroxyprogesterone (Sigma Product No. H-5752) 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: 250, 125, 63, 31 and 15 pg/0.1 ml.
- (B) **Dilution buffer:** 0.05 M Tris-HCl (Sigma Product No. T-3523), pH 8.0, containing 0.1 M NaCl, 0.1% gelatin (Sigma Product No. G-2500) and 0.1% sodium azide.
- (C) **Dextran coated charcoal suspension:** 1.0% activated charcoal untreated powder 100-400 mesh (Sigma Product No. C-5260), 0.1% dextran approximate average molecular weight 70,000 (Sigma 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 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.

RIA Sensitivity

Sensitivity is defined as the 90% intercept of a B/B₀ standard curve. In the above system the sensitivity has been found to be 5 pg.

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
Androstenedione	< 0.01
Corticosterone	< 0.01
Cortisol	< 0.01
Estradiol	< 0.01
Estriol	< 0.01
Estrone	< 0.01
20 α -Dihydroprogesterone	< 0.01
5 α -pregnane-3,20-dione	0.20
Progesterone	2.50
Testosterone	< 0.01

RIA Affinity Constant

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

$$K_a = 1.9 \times 10^{10} \text{ L/mole.}$$

17 α -Hydroxyprogesterone Levels

1. Men	31-317 pg/0.1ml
2. Women	
Follicular Phase	20-72 pg/0.1ml
Luteal Phase	35-221 pg/0.1ml
Pregnant (12th week to term)	1289-2180 pg/0.1ml

Bibliography

1. Kohen, F., et al., in Steroid Immunoassays (Cameron, E.H.D., et al., eds., Alpha Omega Publ., Cardiff, Wales), p. 11 (1975).
2. Youssefnejadian, E., et al., Steroids, **20**, 773 (1972).

* Each vial contains no more than 20 mg Polyvinylpyrrolidone (PVP). Due to the PVP 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.

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