

Product Information

ANTI-DIHYDROTESTOSTERONE-1-BSA

Developed in Rabbit
Whole Antiserum

Product No. **D 3062**

Product Description

The antiserum is developed in rabbit using 5 α -dihydrotestosterone-1-carboxyethyl thioether-BSA as the immunogen. The product is provided as antiserum with 0.1% sodium azide.*

Product Profile

Antibody titer: 1:1,000

Storage

For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used 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 undiluted antiserum has been found to bind at least 40% of 5-10 picograms of tritiated (³H) 5 α -dihydrotestosterone with a specific activity of approximately 100 Ci/mmol as tracer.

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 :g/ml 5 α -Dihydrotestosterone (Product No. A8380) in absolute ethanol. Dilute a portion of the stock solution with buffer (B) to a concentration of 5 ng/ml. This is further diluted in buffer (B) to obtain standard solutions at the following concentrations: 500, 250, 125, 63, 31, 15, 8, and 4 pg/0.1 ml.

- (B) Dilution buffer: 0.05 M Tris-HCl (Product No. T3253), pH 8.0, containing 0.1 M NaCl, 0.1% Gelatin (Product No. G2500) and 0.1% sodium azide.
- (C) Dextran coated charcoal suspension: 1.0% activated charcoal untreated powder 100-400 mesh, 0.1% Dextran approximate average molecular weight 70,000 (Product No. 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. 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.

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
5 ∇ -Androstane-3 ∇ ,17 \exists -diol	18.0
5 ∇ -Androstane-3 \exists ,17 \exists -diol	11.0
Androstenedione	0.1
Androsterone	0.1
Cortisol	< 0.1
Dehydroepiandrosterone	< 0.1
5 \exists -Dihydrotestosterone	4.0
17 \exists -Estradiol	< 0.1
Epiandrosterone	< 0.1
17 ∇ -Epiandrosterone	1.4
Progesterone	< 0.1
Testosterone	44.0

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 10 pg/tube.

RIA Affinity Constant

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

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

5 α -Dihydrotestosterone Levels

1. Men 25-46 pg/0.1 ml
2. Women 13.5-28 pg/0.1 ml

Bibliography

1. Bauminger, S., et al., Steroids, **24**, 477 (1974).
2. Tremblay, R.R., et al., Steroids, **16**, 29 (1970).

Precautions and Disclaimer

Due to the 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.

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