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## TECHNICAL INFORMATION

# ImmunO™

Catalog Number: 613401, 613402, 613404

### Anti-5alpha-Dihydrotestosterone-1-BSA Serum (Rabbit)

**Antigen:** 5-a-dihydrotestosterone-1-carboxyethyl thioether bovine serum albumin was used for immunization.

**Working Dilution:** Reconstitute with 0.5 ml of buffer. This is the stock antiserum solution. A 1:100 dilution of the stock antiserum yields the working dilution.

**RIA Performance:** Dextran coated charcoal RIA method using 5-10 pg/tube tritiated 5-a-dihydrotestosterone with specific activity of approximately 100 ci/mole as tracer and 0.5 ml of properly diluted antiserum.

**Sensitivity:** 10 pg/tube.

**Antibody Affinity Constant KA:**  $5.8 \times 10^9$  L/M (Scatchard Plot)

#### **Specificity:** Steroid% Cross-Reactivity

5alpha-dihydrotestosterone 100  
5alpha-Androstane-3,17-dione 17.14  
Testosterone 22.7  
Androstenedione 2.4  
Androsterone <0.01  
Epiandrosterone 0.80  
5alpha-androstane-3alpha,17beta-diol 0.06  
5alpha-androstane-3beta,17beta-diol 0.05  
Dihydroepiandrosterone <0.01  
Estradiol-17beta <0.01  
Progesterone 0.75  
Cortisol 0.01

### Dextran Coated Charcoal Radioimmunoassay for Rabbit Anti-5alpha-Dihydrotestosterone Serum

#### **Reagents:**

1. Buffer: 0.05M Tris-HCl buffer, pH 8.0 containing 0.1M NaCl, 0.1% sodium azide, and 0.1% gelatin.
2. Dextran-coated charcoal: 0.5% w/v Norit A activated charcoal and 0.05% w/v Dextran T-70 in buffer.
3. Standards: Prepare a standard solution of 1 ug/ml 5alpha-dihydrotestosterone in absolute ethanol. Dilute an aliquot to a concentration of 5 ng/ml. Five serial doubling dilutions are prepared in ethanol from the 5 ng/ml standard giving the following standard solutions:  
5.0 ng/ml 0.63 ng/ml  
2.5 " " 0.31 " "  
1.2 " " 0.15 " "
4. Antiserum: Working dilution of anti-5alpha-dihydrotestosterone serum in assay buffer.
5. Radiolabeled 5alpha-dihydrotestosterone: Prepare the tritiated steroid at 50,000-100,000 dpm/ml at specific activity of 100 Ci/mmol for use as tracer.
6. Scintillant: We recommend the use of MP's Ecolume scintillation cocktail (code no. 882475). This is an environmentally safe cocktail and is excellent for samples from 0-30% aqueous volume as well as for non-aqueous applications.

#### **RIA Method:**

1. Pipette in duplicate 0.1 ml of standards to assay tubes. Prepare a zero control, a blank, and total tubes.
2. Add 0.5ml antibody to all tubes except total and blank tubes. To these add 0.5 ml of buffer.
3. Incubate all tubes at room temperature for 30 minutes.

4. Add 0.1 ml of tritiated steroid to all tubes and incubate at 37°C for 60 min. or overnight at 4°C.
5. Cool at 4°C for 15 minutes.
6. Add at 4°C 0.2 ml dextran coated charcoal solution to each tube excluding the total tube to which 0.2 ml of buffer is added.
7. Mix all tubes on a vortex mixer, incubate at 4°C for 10 minutes, and centrifuge at 4°C at 3000 rpm for 15 minutes.
8. Remove 0.25 ml of supernatant and add to 3 ml of scintillation fluid. Count in liquid scintillation spectrometer.

**Calculations:**

1. Convert all counts to counts per minute (cpm) and average the duplicates for the zero control, blank, total, standards, and samples.
2. Correct all of the above count rates by subtracting the nonspecific binding blank.
3. Calculate the percent bound (%B) for standards and samples.

$$\%B = \frac{\text{Corrected cpm for standard or sample}}{\text{Corrected zero control}} \times 100$$

4. On semi-logarithmic graph paper or on logit paper, plot the %B for each standard against the log-dose of standard (pg of steroid added).

**5alpha-Dihydrotestosterone Levels (1,2):**

Males (normal) 250-460 pg/ml

Females (normal) 135-280 " "

**References:**

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

**Note:** This product may contain a preservative such as sodium azide, thimerosal or proclin. Please see lot specific chemical credential for preservative information.

[If a titer/working dilution is not given above, please click here to see a general dilution chart for working with antibodies. Please note that the general dilution chart should only be used as a guideline. Each lab should determine their own optimal working dilution.](#)

[Will this antibody work with your application? Please click here to see a general chart of antibody applications. Please note that any information given above is primary application data. The general applications charts should only be used as a reference.](#)