MP Biomedicals, LLC
29525 Fountain Parkway
Solon, Ohio 44139
Telephone: 440/337-1200
Toll Free: 800/854-0530
Fax: 440/337-1180
web: http://www.mpbio.com

Certificate of Analysis

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

ImmunO™

Catalog #: 61340
Lot #: 7210J

Form: Lyophilized

Immunogen:DHT-3-CMO-BSA

Reconstitution: 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.

Storage: Lyophilized material should be stored at 2-8ºC. After reconstitution, aliquot and store at -20ºC. Working dilution should be discarded if unused within 12 hours. Avoid repeated freeze-thaw cycles.

Specificity: Steroid% Cross-Reactivity

Dihydrotestosterone 100.0 %
Testosterone 75.0
5d-androstane-3b17bdiol 2.0
5d-androstane-3a17bdiol 1.0
Epitestosterone 1.1
Other steroids <0.1

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/ml0.63 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 37ºC for 60 min. or overnight at 4ºC.
5.Cool at 4ºC for 15 minutes.
6.Add at 4ºC 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 4ºC for 10 minutes, and centrifuge at 4ºC 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:

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

Approved by: *Robert Beattie*
Quality Control Director

Control # R0910