

**Product No. A-0795**  
**Lot 116H4880**

**Anti-Androstenedione**  
Developed in Rabbit  
Whole Antiserum

Antiserum was developed in rabbit using androstenedione-7 $\alpha$ -BSA as the immunogen. The product is diluted antiserum that has been lyophilized.\*

### Reconstitution

To one vial of lyophilized powder, add 5.0 ml of 0.05 M Tris-HCl buffer, pH 8.0, containing 0.1 M NaCl, 0.1% gelatin and 0.1% sodium azide. Rotate gently until the powder dissolves. This is the stock antiserum solution. To obtain the number of tests indicated on the vial, the reconstituted antiserum should be further diluted 10-fold with the same buffer to produce the working dilution of the antiserum.

### Storage

Prior to reconstitution, store at 2-8°C. After reconstitution, the solution may be frozen in working aliquots. The working dilution of the antiserum should be discarded if unused within 12 hours. Repeated freezing and thawing is **not** recommended.

### Tests per Vial

The number of tests per vial is determined at Sigma 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 15-20 picograms of tritiated androstenedione with a specific activity of approximately 50 ci/mmol.

The number of tests per vial and subsequent lot specific data indicate the performance of the antiserum in the assay system utilized at Sigma. It is recommended that the antiserum first be evaluated in the particular assay system chosen due to differences in assay systems and procedures.

### Reagents

A). Standard: Prepare a stock standard solution of 1  $\mu$ g/ml androstenedione (Sigma Product No.

A-9630) in absolute ethanol. Dilute a portion of the stock standard with buffer (B) to a concentration of 1,000 pg/0.1 ml. The 1,000pg/ml standard should be further diluted in buffer (B) to obtain standard solutions at the following concentrations: 31, 63, 125, 250, 500, and 1000 pg/0.1 ml.

- B). 0.05 M Tris-HCl (Sigma Product No. T-3253) buffer, pH 8.0, containing 0.1 M NaCl, 0.1% gelatin (Sigma Product No. G-2500), and 0.01% sodium azide.
- C). Dextran coated charcoal suspension: Buffer (B) containing 0.5% activated charcoal untreated powder 250-350 mesh (Sigma Product No. C-5260) and 0.05% dextran approximate average molecular weight 70,000 (Sigma Product No. D-1390). It is important that the dextran be in solution before the addition of charcoal. The suspension should be stirred and kept at 0°C in an ice-water bath 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 antiserum reconstituted and diluted in buffer (B) to the working dilution.
2. Vortex the tubes.
3. Incubate at room temperature for 30 minutes.
4. Add 0.1 ml tritiated radioactive tracer prepared fresh in buffer (B).
5. Vortex the tubes.
6. Incubate for 60 minutes at 37°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.

## Specificity

Specificity of the antiserum is defined as the ration of antigen concentration to cross-reactant concentration at 50% inhibition of maximum binding. The cross-reactivity data obtained in Sigma's dextran coated charcoal tritiated RIA system is as follows:

Cross-Reactant	% Cross Reactivity
5 $\alpha$ -Androstane-3,17-dione	67
Dihydroepiandrostenedione	6
11-Deoxycorticosterone	<0.001
17 $\beta$ -Estradiol	<0.001
Estrone	<0.001
Progesterone	<0.001
Testosterone	4.5

## Sensitivity

Sensitivity is defined as the 90% intercept of a B/B<sub>0</sub> standard curve. Using Sigma's RIA system, the sensitivity has been found to be 10 pg per tube.

**Affinity Constant:**  $K_a = 3.0 \times 10^9$  L/Mole

## Androstenedione Levels<sup>2</sup>

	pg/0.1ml
1. Men	60-180
2. Women	70-310
(A) Postmenopausal	50-130
(B) Ovariectomized	40-90

\* Each vial contains no more than 20 mg Polyvinylpyrrolidone (PVP). 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.

## References

1. Weinstein, A., et al., Steroids, **20**, 789 (1972).
2. Thorneycroft, I., et al., Steroids, **21**, 111 (1973).