ProductInformation

Anti-Aldosterone
Developed in Rabbit
Whole Antiserum

Product No. **A 3793**
Lot 081H4834

The antiserum is developed in rabbit using aldosterone-3-(O-Carboxymethyl)-oxime as the immunogen. The product is provided as a pre-diluted antiserum that has been lyophilized.

Reconstitution and Dilution

1. Stock Solution: To one vial of lyophilized powder add 5.0 ml of 0.05M potassium phosphate buffered saline, pH 7.4, containing 0.5% BSA 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 5-10 picograms of tritiated (^{3}H) aldosterone with a specific activity of approximately 100 Ci/mmole.

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 1µg/ml aldosterone (Sigma Product No. A 6628) in absolute ethanol. Dilute a portion of the stock solution with buffer (B) to a concentration of 500 pg/0.1ml. This is further diluted in buffer (B) to obtain standard solutions at the following concentrations: 250, 125, 63, 31, 15, and 7.5 pg/0.1ml.

(B) Dilution buffer: 0.05M potassium phosphate buffered saline, pH 7.4, 0.5% BSA (Sigma Product No. A 7030) 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 0.1ml sample or standard (A) and 0.5ml diluted antiserum.
2. Vortex the tubes.
3. Incubate for 30 minutes at room temperature.
4. Add 0.1ml tritiated radioactive tracer diluted in dilution buffer (B).
5. Vortex the tubes.
6. Incubate for 18-20 hours at 4 °C.
7. Rapidly add 0.2ml cold dextran coated charcoal suspension (C) to each tube.

8. Vortex the tubes.
9. Incubate for 10 minutes at 0 °C in ice-water.
10. Centrifuge at 2000 x g for 15 minutes at 4 °C.
11. 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/B_0 standard curve. In the above system the sensitivity has been found to be 5pg/tube.

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**
Allo-tetrahydro-Compound A (allo-THA)	<0.01
Allo-tetrahydro-Compound S (allo-THS)	<0.01
Allo-tetrahydro-Corticosterone (allo-THB)	<0.01
Allo-tetrahydro-Cortisol (allo-THF)	<0.01
Allo-tetrahydro-Cortisone (allo-THE)	<0.01
Cortisol	<0.01
17-Iso-Aldosterone	<0.1
Progesterone	<0.01
Testosterone	<0.01

RIA Affinity Constant
The affinity constant (K_a) is determined by a Scatchard plot using the described RIA system.
$K_a = 1 \times 10^{10}$ L/mole.

Aldosterone Levels[1,2,3]

1. Plasma

Men	4.9-25.5	pg/0.1ml
Women	3.4-35.1	pg/0.1ml
Hypoadrenocorticism	2.7	pg/0.1ml
Primary Aldosteronism		
9:30 A.M.	36.0	pg/0.1ml
5:00 P.M.	51.4	pg/0.1ml

2. Urine

Men	5-18 µg/24 hours
Women	6-17 µg/24 hours
Hospitalized Adults	5-28 µg/24 hours

Bibliography

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2. Varsano-Aharon, N. and S. Ulick, J. Clin.Endocr. Metab., **37**, 372 (1973).
3. Adlercreuts, H., in Methods of Hormone Analysis, (Breuer, H., et al., eds., John Wiley and Sons, New York), p. 236 (1976).