SIGMA CHEMICAL COMPANY

Product No. A-3668
Anti-Angiotensin I
Developed in Rabbit

Lot 083H48571

This antiserum is developed using angiotensin I-Asp[1]-Ile[5]-BSA as the immunogen. The product is provided as a lyophilized, pre-diluted antiserum.*

Reconstitution Instructions

To one vial of lyophilized powder, add 5.0 ml sodium phosphate buffer (0.15 M, pH 7.5) containing 0.03 M ethylenediaminetetraacetic acid (EDTA) disodium salt, 0.1% human serum albumin (HSA), and 0.1% sodium azide. Heat the buffer for 30 minutes at 56°C and cool to room temperature prior to use in reconstituting the antiserum in order to inactivate any proteolytic enzymes that might be present in the HSA. 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 0-5°C. After reconstitution, the stock solution should be separated into aliquots and frozen. 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 40% of 10 picograms of iodinated angiotensin I with a specific activity of approximately 1,000 μCi/μg.

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 µg/ml Angiotensin I acetate salt, human form, synthetic (Sigma Product No. A-9650) in deionized water. Keep frozen aliquots at -20°C. Thaw only one sample for each assay. Calibration of the standards against the international research standard of MRC is recommended[3]. Prior to the assay dilute an aliquot of the stock solution in diluent (D) to obtain the 5,000 pg/ml standard. The 5000 pg/ml standard should be further diluted in diluent (D) to give standard solutions at the following concentrations: 20, 40, 80, 160, 310, 620, 1,250, 2,500 and 5,000, pg/ml.

(B) Sodium phosphate buffer (0.15 M, pH 7.5) containing 0.03 M EDTA disodium salt (Sigma Product No. ED2SS), 0.1% HSA (Sigma Product No. A-1887), and 0.1% sodium azide. Heat the buffer at 56°C for 30 minutes and cool to room temperature to inactivate any proteolytic enzymes that might be present in the HSA.
Note: Before addition of the HSA put aside a sample of buffer which will be used for preparation of the charcoal suspension (E).

(C) Acidic inhibitor: Prior to use, mix equal volumes of 0.76 M HCl in deionized water and 0.07 M 8-hydroxyquinoline in ethanol.

(D) Diluent[1,2]: 2.5% v/v of acidic inhibitor in buffer B.

(E) Dextran coated charcoal suspension: Buffer (B) without HSA while also containing 1.25% activated charcoal untreated powder 100-400 mesh (Sigma Product No. C-5260) and 0.25% dextran approximate average molecular weight 70,000 (Sigma Product No. D-1390). It is important that the dextran be in solution before addition of the charcoal and that the dextran coated charcoal suspension 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:
   0.2 ml sample or standard (A), 0.5 ml antiserum reconstituted and diluted in buffer (B) to the working dilution, and 0.1 ml l-125 radioactive tracer prepared fresh in buffer (B).
2. Vortex the tubes.
3. Incubate for 18-20 hours at 4°C.
4. With tubes at 0°C in ice/water rapidly add 0.4 ml cold dextran coated charcoal suspension (E) to each tube.
5. Vortex the tubes.
6. Centrifuge at 2000 x g for 15 minutes at 4°C.
7. Remove supernatant from each tube and determine the amount of radioactivity present in the supernatant.

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 Sigma's dextran coated charcoal l-125 RIA system is as follows:

Cross-Reactant	% Cross-Reactivity
Angiotensin II	< 0.01
Angiotensin III	< 0.01
Asn^1-Val^5-Angiotensin I	18

Sensitivity

Sensitivity is defined at the 90% intercept of a B/B_O standard curve. Using Sigma's RIA system the sensitivity has been found to be 4 pg per tube.

Affinity Constant

The affinity constant (K_a) is determined by a Scatchard plot using Sigma's RIA system.
$K_a = 3.0 \times 10^{10}$ L/mole.

Values for Plasma Renin Activity in Healthy and Diseased Subjects[4]

Condition	Daily sodium intake (mmole)	PRA (µg/l/hour) Supine	PRA (µg/L/hour) Upright
1.Control patients	100-140	1.34 ± 0.27	3.09 ± 0.41
2.Essential hypertension	100-140	1.21 ± 0.08	1.92 ± 0.17
3.Primary aldosteronism	100-140	0.26 ± 0.04	0.35 ± 0.05
4.Renovascular hypertension	100-140	9.17 ± 1.48	19.5 ± 3.23
5.Anephric (males)	80	0.0 - 0.08 (range)	

Bibilography

1. Morris, B.J., Clinica. Chimica. Acta, **75**, 503 (1977).
2. Vader, H.L., et al., Clinical. Chimica. Acta, **75**, 253 (1977).
3. Bangham, D.R., et al., Clin. Sci. and Molec. Medicine, **48**, 135 (1975).
4. Fhyrquist, F., et al., Clin. Chem., **22**, 250 (1976).

* Each vial contains no more than 20 mg Polyvinylpyrrolidone (PVP). Due to the PVP 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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