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ProductInformation

Anti-Angiotensin I
produced in rabbit, whole antiserum

Catalog Number **A3668**

Product Description

Anti-Angiotensin I is developed in rabbit using as immunogen angiotensin I-Asp^{1}-Ile^{5}-BSA. The antibody is provided as a lyophilized, pre-diluted antiserum.

Anti-Angiotensin I recognizes angiotensin I by radioimmunoassay (RIA). The antibody does not cross-react with angiotensin II or angiotensin III by RIA.

Reagent

Anti-Angiotensin I is provided as a lyophilized, pre-diluted antiserum. Each vial contains no more than 20 mg polyvinylpyrrolidone (PVP).

Reagents recommended, but not provided

Angiotensin I human acetate, Catalog Number A9650
EDTA disodium salt, Catalog Number ED2SS
Albumin from human serum, Catalog Number A1887
Dextran, average mol wt 64,000-76,000, Catalog Number D4751

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Prior to reconstitution, store at 2-8 °C. After reconstitution, freeze in working aliquots at –20 °C. Working dilutions should be discarded if not used within 12 hours. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended.

Preparation Instructions

To one vial of lyophilized powder, add 5 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.

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 ~1,000 µCi/µg. The number of tests per vial 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.

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 ^{125}I 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

Procedure

Reagents

1. Standard: Prepare a stock standard solution of 1 µg/mL angiotensin I acetate 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 to obtain the 5,000 pg/mL standard. The 5000 pg/mL standard should be further diluted in Diluent to give standard solutions at the following concentrations: 20, 40, 80, 160, 310, 620, 1,250, 2,500, and 5,000, pg/mL.
2. Buffer: sodium phosphate buffer (0.15 M, pH 7.5) containing 0.03 M EDTA disodium salt, 0.1% HSA, and 0.1% sodium azide.
   Note: Before addition of the HSA, put aside a sample of Buffer to be used for preparation of the Dextran-coated Charcoal Suspension.
   Heat the Buffer with HSA at 56 °C for 30 minutes and cool to room temperature to inactivate any proteolytic enzymes that might be present in the HSA.
3. Acidic Inhibitor: Prior to use, mix equal volumes of 0.76 M HCl in deionized water and 0.07 M 8-hydroxyquinoline in ethanol.
4. Diluent [1, 2]: 2.5% v/v of Acidic Inhibitor in Buffer.
5. Dextran-coated Charcoal Suspension: Use Buffer without HSA from step 2; add 1.25% activated charcoal, untreated powder 100-400 mesh, and 0.25% dextran, average molecular weight ~70,000. 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 , 0.5 mL antiserum reconstituted and diluted in Buffer to the working dilution, and 0.1 ml ^{125}I radioactive tracer prepared fresh in Buffer.
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 Charcoal-Dextran Suspension 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.

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

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

References

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).

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