3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone (800) 325-5832 (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

ANTI-HUMAN ATRIAL NATRIURETIC PEPTIDE (ANP)
Developed in Goat
Whole Antiserum

Product No. **A 5050**

Product Description

The antiserum is developed in goat using α-human polypeptide-glutaraldehyde-KLH as the immunogen. The product is provided as an undiluted antiserum containing 0.1% sodium azide as a preservative.

Precautions and Disclaimer

Due to the 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.

Storage

For continuous use, store at 2-8 °C. For extended storage, the solution may be frozen in working aliquots. Repeated freezing and thawing is **not** recommended. Storage in "frost-free" freezers is **not** recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

References

1. Hartter, E., et al., Clin. Chem., **32**, 441 (1986).
2. Yandle, T.G., et al., J. Clin. Endocrinol. Metab., **63**, 72 (1986).
3. Yamaji,, T., et al., Clin. Invest., **76**, 1705 (1985).
4. Burnett, J.C., et al., Science, **231**, 1145 (1986).
5. Sagnella, G.A. and G.A. MacGregor, Trends in Bioch. Sci., **11**, 299 (1986).
6. Fluegge, G., et al., Histochemistry, **86**, 479 (1983).

RIA SYSTEM

RIA Characterization

The antiserum is characterized utilizing the following second antibody-polyethylene glycol (PEG) RIA protocol, where 0.1 ml of antiserum at the working dilution has been found to bind at least 40% of 10 picograms of iodinated α-ANP with a specific activity of approximately 2000 Ci/mmole.

It is recommended that the antiserum first be evaluated in the particular assay system chosen due to differences in systems and procedures.

RIA Dilution Instructions

The working dilution is determined to be at least 1:500, dilute the antiserum in 0.01 M phosphate buffered saline, pH 7.4, containing 0.3% BSA, 0.1% Triton X100, 0.001 M EDTA and 0.1% sodium azide.

RIA Reagents

1. Buffer 1: 0.01 M PBS, pH 7.4 containing 0.3% BSA, 0.1% Triton X100, 0.0001M EDTA and 0.1% sodium azide.
2. Buffer 2: 0.01 M ammonium acetate, pH 5.0.
3. Buffer 3: 0.01 M PBS, pH 7.4.
4. EDTA solution: Ethylenediaminetetraacetic acid (EDTA) disodium salt (Product Code ED2SS), 0.1M, in distilled water. Adjust pH with to 8.6 with 10 N NaOH.
5. Normal Goat Serum: 1% (v/v, Product No. G 9023) in buffer 3.
6. PEG solution: 6% PEG (Product No. P 2139, approximate molecular weight 8,000) in dilution buffer without BSA.
7. Standards: Prepare a solution of 1 mg/ml α-ANP in buffer 2. Dilute an aliquot to 25ng/ml in buffer 1 and then further dilute in buffer 1 to obtain the following concentrations: 12.5, 6.25, 3.2, 1.6, 0.8, 0.4, 0.2, and 0.1 ng/ml.
8. Second antibody: Donkey anti-Goat IgG (Product No. G 8267), reconstituted in buffer 3. Dilute reconstituted antiserum 1:25 in dilution buffer for use.
9. Radiolabelled Tracer: Freshly prepared solution of 100-150 pg/ml ^{125}I-α-ANP in buffer 1 with specific activity of approximately 2000 Ci/mmole.

RIA Protocol

1. In polypropylene test tubes add 0.1ml sample or standard and 0.1ml of antiserum diluted to the working dilution.
2. Vortex the tubes.
3. Incubate for 30 minutes at 4 °C.
4. Add 0.1 ml I^{125} radioactive tracer to all tubes.
5. Vortex the tubes.
6. Incubate for 18-24 hours at 4 °C.

7. Add 0.1 ml EDTA solution to all tubes.
8. Vortex the tubes.
9. Add 0.1 ml diluted goat serum to all tubes.
10. Vortex the tubes.
11. Add 0.1 ml of second antibody to all tubes.
12. Vortex the tubes.
13. Add 0.5 ml of PEG solution to all tubes.
14. Vortex the tubes.
15. Incubate at room temperature for 10 minutes.
16. Centrifuge at 3000 x *g* for 15 minutes at 4 °C.
17. Remove supernatant from each tube and determine the amount of radioactivity present in the precipitate.

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 20 pg/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 second antibody-PEG I^{125} RIA system is as follows:

Cross-Reactant	**%Cross-Reactivity**
Human ANP Fragment 3-28	< 50
Human ANP Fragment 4-28	< 50
Rat ANP	< 50
Rat ANP Fragment 3-28	< 80
Atriopeptin I (Rat ANP Fragment 5-25)	<50
Atriopeptin II (Rat ANP Fragment 5-27)	<50
Atriopeptin III (Rat ANP Fragment 5-28)	<50
Vasopressin (Arg^8)	<10
Oxytocin	<10
Somatostatin	<10

RIA Affinity Constant

The affinity constant (K_a) is determined by a Scatchard plot using this RIA system.
K_a = 1-10 x 10^9L/mole.

PCS/KMR 5/02