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ProductInformation

Anti-3′5′-Cyclic Adenosine Monophosphate (cAMP)
Developed in Rabbit, Whole Antiserum

Product No. **A 0670**

Product Description

The antiserum is developed in rabbit using 3′5′-cAMP-2′-BSA as the immunogen. The antibody is provided as a pre-diluted antiserum that has been lyophilized.

3′5′-Cyclic Adenosine Monophosphate (c-AMP) is an intracellular hormonal mediator that was first described as a mediator of the glycogenolytic effect of epinephrine and glucagon in the liver. Since then, it has been found in microorganisms, plants, and animals. The biological effect of c-AMP is mediating the effect of a broad range of hormones, suggesting that c-AMP serves as a universal mediator of hormone stimulation.

Preparation Instructions

1. Stock Solution: To one vial of lyophilized powder, add 1.0 ml of 0.1% BSA in distilled water. Rotate 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/Stability

Prior to reconstitution, store at 2-8 °C. After reconstitution, aliquot and freeze. Repeated freezing and thawing is **not** recommended. Do not store in a frost-free freezer. Working dilutions should be discarded if unused after 12 hours.

RIA Characterization

The antiserum is characterized utilizing the following ethanol precipitation radioimmunoassay (RIA) protocol, where 0.1 ml of reconstituted and diluted antiserum has been found to bind at least 40% of 5 fmole of iodinated cAMP-2′-succinyl-tyrosine methyl ester with a specific activity of approximately 2,000 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

Note: All nucleotide containing solutions should be kept in an ice-water bath, during use.

(A) Standards: Prepare a stock standard solution of 1 mg/ml cAMP free acid (Sigma Product No. A 4137) in distilled water, keep in ice-water bath. Determine the exact concentration by measuring the absorbance at 259 nm (E1% = 15.4). Dilute the stock standard with cold acetate buffer (B) to a concentration of 5000 fmole/0.1 ml. This is then further diluted in cold acetate buffer to obtain the following standard solutions: 2500, 1000, 500, 250, 100, and 50 fmole/0.1 ml
(B) 0.05 M Sodium acetate (Sigma Product No. S 8625) buffer, pH 6.2.
(C) BSA Solution 1: 0.1% BSA (Sigma Product No. A 7030) in distilled water.
(D) BSA Solution 2: 10% BSA (Sigma Product No. A 7030) in distilled water.
(E) Succinylation Reagent: Dissolve 200 mg succinic anhydride (Sigma Product No. S 7626) in 1 ml dry acetone. Add triethylamine at a ratio of 25:9 (v/v) succinic anhydride:triethylamine.

Succinylation of Samples and Standards

1. In polypropylene test tubes, add 0.1 ml sample or standard and 0.02 ml fresh succinylation reagent.
2. Vortex the tubes and place in ice-water bath.
3. Add 1.9 ml cold acetate buffer to each tube.
4. Vortex the tubes and keep in ice-water bath.

RIA Protocol

1. In polypropylene test tubes add 0.1 ml succinylated sample or standard (A) and 0.1 ml diluted antiserum.
2. Vortex the tubes.
3. Incubate for 4 hours in ice-water bath.
4. Add 0.1 ml iodinated radioactive tracer diluted in acetate buffer (B).

5. Vortex the tubes.
6. Incubate for 18-20 hours at 2-8 °C.
7. Add 0.1 ml BSA solution 2 to each tube.
8. Vortex the tubes.
9. Add 2 ml cold ethanol to each tube.
10. Vortex the tubes.
11. Centrifuge at 2000 x g for 15 minutes at 2-8 °C.
12. Remove supernatant from each tube 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 5 fmole/tube.

RIA Affinity Constant
The affinity constant (K_a) is determined by a Scatchard plot using the described RIA system.
K_a = 1-10 x 10^9 l/mole.

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**
5r-Adenosine Monophosphate	<0.001
5r-Adenosine Diphosphate	<0.001
5r-Adenosine Triphosphate	<0.001
3′5′-cGMP	<0.001

References

1. Frandsen, E.K. and G. Krishna, Life Sciences, **18**, 529 (1977).

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