

**Product No. A 0670**  
**Lot 087H4856**

**Anti-3':5'-Cyclic Adenosine Monophosphate (cAMP)**  
Developed in Rabbit  
Whole Antiserum

The antiserum is developed in rabbit using 3':5'-cAMP-2'-BSA 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 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**

Prior to reconstitution store at 2-8°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 ethanol precipitation radioimmunoassay (RIA) protocol, where 0.1 ml of reconstituted and diluted antiserum has been found to bind at least 40% of 6 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 at 0°C 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 at 0°C in ice-water bath. Determine the exact concentration by measuring the absorbance at 259nm ( $E_{259}^{1\%} = 15.4$ ). 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 (0°C).
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 at 0°C.
4. Add 0.1 ml iodinated radioactive tracer diluted in acetate buffer. (B).

5. Vortex the tubes.
6. Incubate for 18-20 hours at 4 °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 4 °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<sub>0</sub> standard curve. In the above system the sensitivity has been found to be 5 fmole/tube.

### RIA Affinity Constant

The affinity constant (K<sub>a</sub>) is determined by a Scatchard plot using the described RIA system.  
K<sub>a</sub> = 1.1 x 10<sup>11</sup> 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 |
|----------------------------|-------------------|
| 5'-Adenosine Monophosphate | <0.001            |
| 5'-Adenosine Diphosphate   | <0.001            |
| 5'-Adenosine Triphosphate  | <0.001            |
| 3':5'-cGMP                 | <0.001            |

### Bibliography

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