# ProductInformation

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

Product No. **A0670**
Lot No. 097H4805

The antiserum is developed in rabbit using 3ρ:5ρ-cAMP-2ρ-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 2-8EC.
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-2ρ-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 0EC 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 0EC 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. S8625) buffer, pH 6.2.
(C) BSA Solution 1: 0.1% BSA (Sigma Product No. A7030) in distilled water.
(D) BSA Solution 2: 10% BSA (Sigma Product No. A7030) in distilled water.
(E) Succinylation Reagent: Dissolve 200 mg succinic anhydride (Sigma Product No. S7626) 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 (0EC).
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 0EC.
4. Add 0.1 ml iodinated radioactive tracer diluted in acetate buffer. (B).

5. Vortex the tubes.
6. Incubate for 18-20 hours at 4EC.
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 4EC.
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.
$K_a = 1.1 \times 10^{11}$ 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** |
|---|---|
| 5ρ-Adenosine Monophosphate | <0.001 |
| 5ρ-Adenosine Diphosphate | <0.001 |
| 5ρ-Adenosine Triphosphate | <0.001 |
| 3ρ:5ρ-cGMP | <0.001 |

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

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