

## Extraction and Acetylation Procedure for Cyclic AMP

Includes: Cyclic AMP

Cat #: EA 50

### Materials Needed:

1. 0.4 N HClO<sub>4</sub>
2. 1 N KOH
3. Dowex 1x2 column (0.5x4 cm, Chloride form)
4. Deionized Water
5. 0.5 N HCl
6. Acetic Anhydride
7. Triethylamine

### Reagents Needed:

1. 10 mM PBS, pH 7.5
2. 2:5 Acetic Anhydride:Triethylamine (AA:TEA)

### Extraction Procedure:

1. Tissue (100-150 mg) or cells (1-10x10<sup>6</sup> cells) are homogenized in 1 mL of 0.4 N HClO<sub>4</sub> and then centrifuged at 20,00 x g for 10 minutes
2. The supernatant is neutralized with 1N KOH to pH 7.0-7.5 and then applied to a Dowex 1-x2 column (0.5x4 cm, Chloride form).
3. The column is washed with 5mL of H<sub>2</sub>O.
4. Cyclic AMP is eluted with 3 mL of 0.5 N HCl.
5. This fraction is lyophilized and then dissolved in a suitable volume of standard buffer (10mM PBS buffer pH 7.5).
6. An aliquot of each fraction is acetylated and assayed according to the following procedure.

### Acetylation Procedure:

Note: To increase the sensitivity of the assay, samples and standards must be acetylated following the procedure listed below. The samples and standards should be acetylated and assayed at the same time.

1. After making the standards, place clean test tubes in front of each standard and sample. Label them accordingly.
2. Now make a 2:5 Acetic Anhydride (AA), Triethylamine (TEA) mixture. Both are light sensitive. Pipette 750 µL of TEA into a small test tube. Then take 300 µL of Acetic Anhydride and pipette it in the same test tube. **VORTEX IMMEDIATELY!!** TEA must be placed in the test tube first. Vortex for 5 seconds.
3. Take the AA:TEA mixture and pipette 10 µL into all empty test tubes for standards and samples.

4. Starting with the "0" standard (10mM PBS), pipette 300  $\mu$ L into the first test tube with 10  $\mu$ L of AA:TEA mixture. **VORTEX IMMEDIATELY!!** Vortex for 5 seconds.
5. Repeat this procedure for all 8 standards and for each sample. Standards and samples are ready to be assayed immediately following acetylation.