DetectX®

Cyclic Nucleotide Tissue Extraction Protocol

For our DetectX® Cyclic AMP and cGMP Immunoassay Kits

ARBOR ASSAYS®

INTRODUCTION

To extract the cyclic nucleotides, cAMP or cGMP, (cNMPs) from tissue samples we recommend using the Sample Diluent provided in the assay kit. Listed below is our suggested protocol. Use of kit Sample Diluent will also allow protein determination using the DetectX® BCA Protein Detection Assay, K041-H1.

MATERIALS NEEDED

- Sample Diluent, 4x Concentrate, X074-12ML/-60ML, diluted 1:4 by taking one part Sample Diluent Concentrate and adding 3 parts water
- Polypropylene Centrifuge tubes of suitable size for samples
- Homogenizer
- Test tubes for sample supernatant collection
- Cyclic AMP or GMP standard from the kit (for optional recovery efficiency determination)

PROCEDURE

Ensure that the sample is as free from debris and as dry as possible.

1. Weigh up to 50 mg of sample into 50 mL centrifuge tube.
2. Add 0.3 mL diluted Sample Diluent per mg of Sample and homogenize using a hand held or bead homogenizer.
3. Centrifuge the mixture at 13,000 x g for 10 min at 4°C and carefully remove the supernatant to a clean tube.
4. Reserve a portion of supernatant for protein determination if desired.
5. Run sample supernatant and subsequent dilutions as directed in the kit manual.

RECOVERY EFFICIENCY (OPTIONAL)

1. If you wish to determine the efficiency of recovery for your samples, add sufficient cyclic nucleotide to a portion of one sample (Control Sample), leaving a second identical portion unspiked prior to homogenization.
2. Recovery efficiency is determined by comparing the concentration of the cyclic nucleotide measured in the Control sample to the concentration of cyclic nucleotide added (Spike), measured or theoretical, after subtracting the cyclic nucleotide concentration measured in the unspiked sample.

$$\% \text{ Efficiency} = \frac{(\text{cNMP in Control Sample} - \text{cNMP in Unspiked Sample}) \times 100}{\text{cNMP Spike Concentration}}$$

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