**A Stable, Sensitive Fluorescence-Based Method for Detecting Cyclic AMP**

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**Abstract**

Adenosine 3',5'-cyclic monophosphate (cyclic AMP, cAMP) is a universal secondary messenger that connects changes in the extracellular environment to changes in cell functions and human disease, serving as an active inroads to understanding their response and the process of cell dysfunction. This report examines the performance of fluorescence-based competitive immunoassays for cAMP in 96-well microplates. Using purified cAMP as a competitor, the optimal conditions for detection were determined to be 0.1 M phosphate buffer, pH 7.0, and 0.05% Tween 20. Using these conditions, the sensitivity of the assay was determined to be 0.05 pmol/mL, with a limit of detection of 0.1 pmol/mL. The assay was found to be specific to cAMP, with no cross-reactivity to other cyclic nucleotides or related compounds. The assay was found to be reproducible, with intra-assay coefficients of variation ranging from 3% to 5% and inter-assay coefficients of variation ranging from 6% to 8%.

**Introduction**

Cyclic AMP is involved in a wide range of cellular signaling pathways. In many cell types, cyclic AMP is produced by adenyl cyclase and hydrolyzed by cyclic nucleotide phosphodiesterases. The enzyme adenyl cyclase catalyzes the formation of cyclic AMP from ATP. Cyclic AMP functions as a second messenger, binding to specific receptors on the cell surface and acting as a mediator of cellular responses. The assay is designed to detect cyclic AMP in biological samples, including cell extracts and tissue homogenates.

**Materials**

Cyclase: HEX-293 cells chosen for their high levels of adenylate cyclase activity (9). The results of the experiments are shown in Table 1. The assay was found to be sensitive enough to detect cyclic AMP in samples with as low as 0.01 pmol/mL.

**Methods**

Cell preparation: HEX-293 cells were grown in suspension to 10^6 cells/mL in DMEM with 10% FBS and 37°C in 5% CO2. Cells were harvested, washed, and resuspended in DMEM at a concentration of 5 x 10^6 cells/mL. Cells were then incubated for 1 hour at room temperature. The resulting fluorescence intensity was read using Gemini XS and the curve shown in (B) was obtained using Analyst AD.

**Discussion**

Compared to radioactive methods for cAMP detection, such as scintillation proximity assays (SPA) and radiomimetic cAMP, the CatchPoint assay is a stable, sensitive, and specific method for detecting cyclic AMP in cell extracts and tissue homogenates. The assay is designed to detect cyclic AMP in biological samples, including cell extracts and tissue homogenates.

**References**


3. Martin, F., M. J. T., and E. Alcorta. 2001. Mutations affecting the C-terminus of PKA phosphorylate a variety of proteins, which eventually leads to the activation of CREB. This enzyme is critical for basic research and drug discovery purposes. Current methods for detecting cyclic AMP include radiometric assays, which are cumbersome, include indirect costs for waste storage and removal and entail careful handling of radioactive materials. The CatchPoint assay is designed to detect cyclic AMP in biological samples, including cell extracts and tissue homogenates.