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This Week's Citation Classic

Salomon Y, Londos C & Rodbell M. A highly sensitive adenylate cyclase assay. *Anal. Biochem.* **58**:541-8, 1974.
[Section on Membrane Regulation, Lab. Nutrition and Endocrinology, Natl. Inst. Arthritis, Metabolism, and Digestive Diseases, NIH, Bethesda, MD]

This paper describes a technique which permits nearly complete separation (to the level of 2 ppm) of 3'5' cyclic [^{32}P] AMP from an [α^{32}P] ATP, by sequential chromatography on Dowex 50 and neutral alumina. Also described are the necessary procedures for long-term maintenance and reutilization of both types of columns. [The *SCI*® indicates that this paper has been cited over 1,105 times since 1974.]

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When I arrived as a postdoctoral fellow at Mertin Rodbell's laboratory at the National Institutes of Health, adenylate cyclase was assaved by the method of Krishna *et al.*,[1] which separated 3'5' cyclic AMP from other phosphate-containing compounds by sequential chromatography on a cation exchange resin and nascent $BaSO_4$. Although this technique represented a major advance over earlier *in vitro* adenylate cyclase assays, it had several drawbacks. First, the background was too high for accurately measuring true initial reaction rates, a project that Michael Lin and I have begun. Second, differences among lots of [α^{32}P] ATP were reflected in highiy variable background levels which led us occasionally to discard entire experiments. Finally, in order to minimize the problems with high ^{32}P-backgrounds, disposable columns were prepared daily for both the ion exchange and $BaSO_4$ steps. This was a costly and time consuming aspect of the assay.

The above difficulties with the existing technique stimulated a search for a method that would permit better separation of 3'5' cyclic AMP from ATP and impurities. This I accomplished by combining the Dowex cation exchange step of the Krishna method[1] with the neutral alumina chromatographic procedure developed by White and Zenser[2] and Ramachandran.[3] The results obtained with the double chromatographic technique were even better than I had hoped for, since assay backgrounds were reduced to a level barely distinguishable from the machine background of the scintillation counter.

"Another postdoctoral fellow, Constantine Londos, and I then carried out a series of experiments in which we compared this new method with others in use at the time. The results prompted us to write the article which appeared in *Analytical Biochemistry*.

"Given the overwhelming acceptance of this method among investigators in the adenylate cyclase field, we are now pleased to have overcome several obstacles that nearly prevented its publication.

"First, as young postdoctoral fellows, we were unsure as to whether or not such a finding merited publication. Since Rodbell was abroad for several months, we consulted other colleagues who advised us not to waste time on a methods paper. Nevertheless, we could not avoid the feeling that many would welcome an efficient new method free of the problems mentioned above. Moreover, another aspect of the method proved to be a considerable relief. We found that both Dowex and alumina columns could be used repeatedly, without affecting the quality of the results. Additionally, columns once used could be set aside for weeks; the dried residue functions well upon renewal with the described technique. The notion that this labor-saving aspect, coupled with the extremely high sensitivity of the method, would be welcome led us after all to write the article. Finally, it is rather amusing that, in its present form, the paper was initially rejected for insufficient advancement. However, our persistence with the editor resulted in its acceptance.

"An up-to-date cookbook version of this method has recently appeared in *Advances in Cyclic Nucleotide Research."* [4]

1. **Krishna G, Weiss B & Brodie B B.** A simple, sensitive method for the assay of adenyl cyclase.*J. Pharmacol. Exp. Ther.* **163**:379-85, 1968.
[The *SCI* indicates that this paper has been cited over 1,215 times since 1968.]
2. **White A A & Zenser T V.** Separation of cyclic 3',5'-nucleoside monophosphates from other nucleotides on aluminum oxide columns: application to the assay of adenyl cyclase and guanyl cyclase. *Anal. Biochem.* **41**:372-96, 1971. [The *SCI* indicates that this paper has been cited over 240 times since 1971.|
3. **Ramachandran J.** A new simple method for separation of adenosine 3'.5'-cyclic monophosphate from other nucieotides and its use in the assay of adenyl cyclase. *Anal. Biochem.* **43**:227-39, 1971. [The *SCI* indicates that this paper has been cited over 265 times since 1971.]
4. **Salomon Y.** Adenylate cyclase assay. *Advan. Cyclic Nucl. Res.* **10**:35-55, 1979.