

cAMP RIA

Method:	Radio-ImmunoAssay
Vendor:	N/A (In-House protocol/reagents).
Description:	cAMP in plasma or urine, or in cultured cell lysates is measured using an in-house radiometric immunoassay. Users may stimulate their target cells in 96-well plates with test drugs, likely in a buffer containing IBMX to amplify/stabilize the cAMP signal, for a chosen time (e.g. 30 minutes), and then will lyse the cells by replacing the buffer with 50 ul of 50 mM HCl and embedding the plate in powdered dry ice to freeze the contents. The plate is then transferred to the Core for RIA. After thawing and diluting the well contents 5- fold, the Core will analyze an aliquot of the lysate using Core-provided reagents and materials, including 125I-cAMP, anti-cAMP primary antibody, secondary antibody, and 96-well RIA "strip" plates). Users may also choose to work more directly with assistance of a Core technician to perform the cell manipulations. The Core's 10-channel TiterTek gamma counter is used for data acquisition, and the Core will assist in data calculation and graphing analyses, as needed. Costs are based on use of radioisotope and assay reagents provided by the Core.

Collection and Performance Characteristics

Tube type:	Plasma and urine tubes or 96 well plates. Cultured cells in 96-well plates, treated with test agent and frozen per instructions above. Samples should be delivered frozen.
Sample Volume:	1 to 50 ul
Reference range:	0.03 – 250 fmol/ul
Notes:	Contact Core to arrange sample submission and assay.

1

Director: Thomas Gardella, Ph.D.
 Braden Corbin, Technician
 Email: gardella@helix.mgh.harvard.edu Website:
<https://csr.mgh.harvard.edu/cell-signaling>