Bridge-It™ cAMP Fluorescence Assay

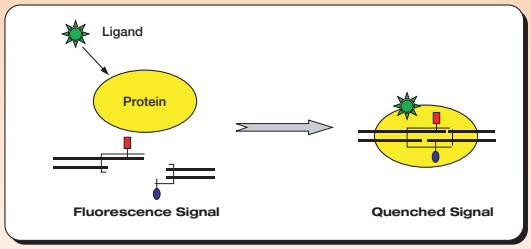
Easy - Fast - Sensitive - Flexible
Suitable for Use With Adherent and Non-Adherent Cells
Adaptable to Low- and High-Throughput Testing Formats

Sequence-Specific DNA Binding Proteins

Eukaryotic cells contain an estimated 3,000 sequence-specific DNA binding proteins. These important proteins, acting either with or without a specific small molecule co-regulator (ligand), control all aspects of genomic DNA activity including gene expression, DNA replication, and DNA repair. Mediomics is applying its novel fluorescence assay platform to develop assays useful for rapidly and sensitively quantifying the activity of both DNA binding proteins and their small molecule ligands.

New Fluorescence Platform For Measuring Sequence-Specific DNA Binding Proteins And Their Ligands

The common property of all sequence-specific DNA binding proteins is their ability to bind with high affinity and specificity to a DNA duplex containing a unique nucleotide sequence, i.e., the DNA binding site for the protein. Mediomics' assay platform relies on this common characteristic. A DNA duplex containing the sequence-specific DNA binding site for a given target protein is split into two DNA "half-site" duplexes each having a short single-stranded overhand. These single-stranded extensions are short enough so that in the absence of the target protein little spontaneous re-association occurs. When the target protein is present, however, its high affinity for the full-length DNA sequence will drive the re-association of the two half-site DNA duplexes. This re-association can be sensitively detected by incorporating appropriate fluorescence probes into each of the two DNA half-sites. The presence of the DNA binding protein is detected as a quenching of the fluorescence signal. A simple variation of this basic platform design allows a DNA binding protein to function as a sensitive biosensor for its specific ligand as shown in the schematic.



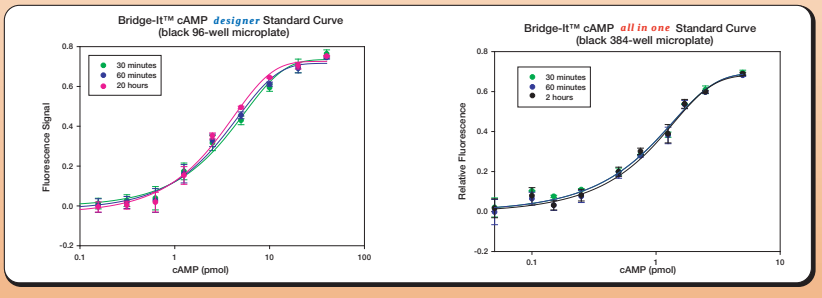
Bridge-It™ Cyclic AMP Assay

Adenosine-3', 5'-cyclic monophosphate (cAMP) is an important second messenger which is involved in the modulation of numerous biological processes. The measurement of cAMP is especially important in new drug discovery since cAMP levels are closely related to the activity of one of the major targets for new drug discovery, the G protein-coupled receptors (GPCR). The Bridge-It™ fluorescence cAMP assay is based on the novel assay platform design described above. Basically CAP protein, a bacterial DNA binding protein whose DNA binding activity depends upon the presence of cAMP, is used as a highly specific biosensor for detecting and measuring cAMP levels in test samples. Mediomics has designed two cAMP products for user flexibility and convenience - the Bridge-It™ cAMP ***designer*** assay and the Bridge-It™ cAMP ***all in one*** assay. These kits may be used to measure cAMP in cultured adherent and non-adherent cells. The Bridge-It™ ***designer*** assay is performed in black 96-well microplates. It is the preferred method for routine laboratory use in research studies that involves cell manipulations such as preparation of pellets, ethanol extraction of cells, or the testing of attached cell monolayers. The Bridge-It™ cAMP ***all in one*** assay is intended to be used with a black 384-well microplate. This design is primarily suited for use by large laboratories that routinely evaluate large numbers of biological test samples or perform high-throughput screening for new drug discovery.

Bridge-It™ cAMP Assays - Selectivity, Sensitivity and Performance Characteristics

The Bridge-It™ cAMP assay is highly specific. ATP, AMP, and cGMP have all been tested for selectivity using the cAMP assay. No response was detected using the Bridge-It™ cAMP assay with any of these compounds within the concentration range that would be expected to occur in "real life" samples (i.e., millimolar range for AMP and ATP, and micromolar range for cGMP). The sensitivity and performance characteristics of the Bridge-It™ cAMP ***designer*** and the Bridge-It™ ***all in one*** assay products are presented in the Table and Figures.

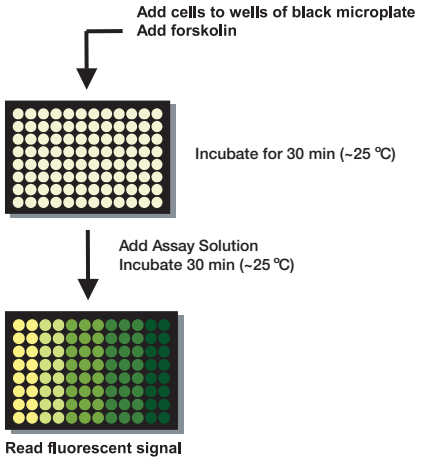
Bridge-It™ cAMP	Black Microplate (recommended)	cAMP Detection Level
designer	96-well	5nM (0.5 pmol/well in 100 µl vol.)
all in one	384-well	5nM (100 fmol/well in 20 µl vol.)

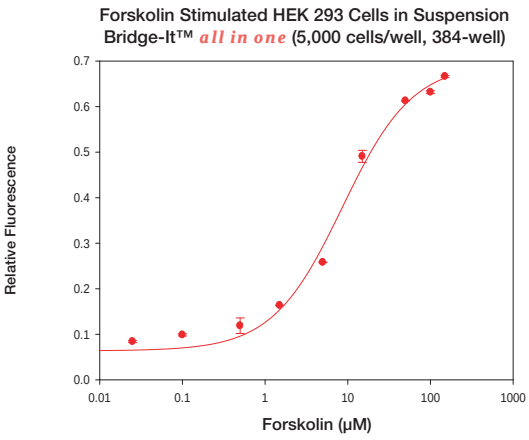


Cell Preparation and Forskolin Stimulation

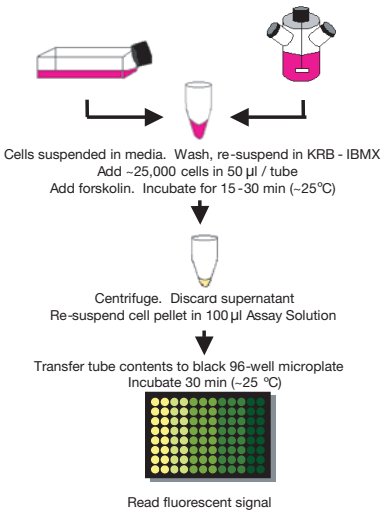
HEK-293 cells were grown using standard cell culture conditions to ~70-80% confluency. The cells were trypsinized, harvested, and washed in serum-free Krebs Ringers Bicarbonate buffer containing a phosphodiesterase inhibitor (i.e., KRB-IBMX buffer). Cyclic AMP (cAMP) levels were measured following forskolin stimulation of the cells using either the Bridge-It™ *all in one* assay or the Bridge-It™ *designer* assay. Following incubation with Bridge-It™ Assay Solution for 30 min at ~ 25°C, the fluorescent signal was read at ~480 nm excitation, ~520 nm emission. Data analysis was performed using relative fluorescence (RF) as a signal change relative to a blank (RF= $(F_o-F)/F_o$). RF analysis has been shown to be highly reproducible and does not depend greatly on the instrument used to read fluorescence. Examples can be seen below.

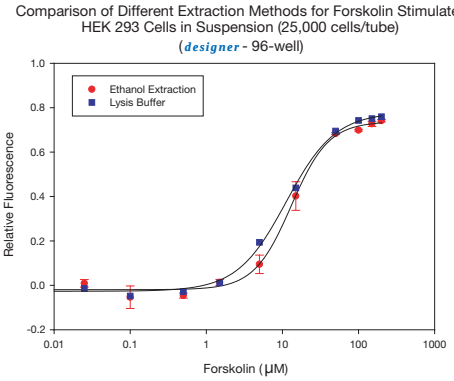
Example of Bridge-It™ cAMP all in one for Cells in Suspension

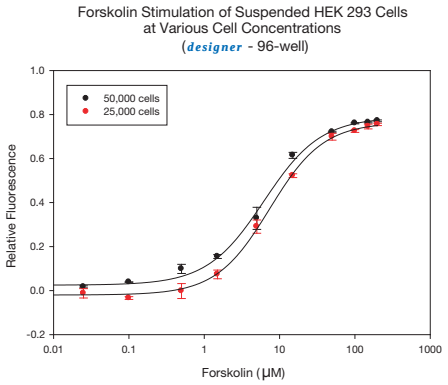




Example of Bridge-It™ cAMP designer Assay for Suspended Cells







Example of Bridge-It™ cAMP for Attached Cells

The Bridge-It™ cAMP *all in one* and *designer* assays also allow for the stimulation and measurement of cAMP in adherent monolayer cells attached to the wells of a tissue culture microplate. In the following example using the *designer* assay, HEK-293 cells were trypsinized and plated into the wells of a 96-well polystyrene tissue culture microplate at a density of 25,000 cells per well in 100 µl. The cells were allowed to attach to the bottom of the wells overnight. The next day, the media was removed and the monolayer was washed with a phosphate buffered saline solution. After the saline solution was replaced with 50 µl KRB - IBMX buffer, forskolin was added to the wells at different concentrations and the microplate was then incubated for 15 minutes at ~25°C. The forskolin containing solution was then replaced with 50 µl of the *designer* Assay Solution and the microplate was incubated for 30 minutes at ~25°C while being gently rotated. Following the incubation, each well content was transferred into a black 96-well microplate and the fluorescent signal was read using the settings for fluoresceine (~480 nm excitation, ~520 nm emission).