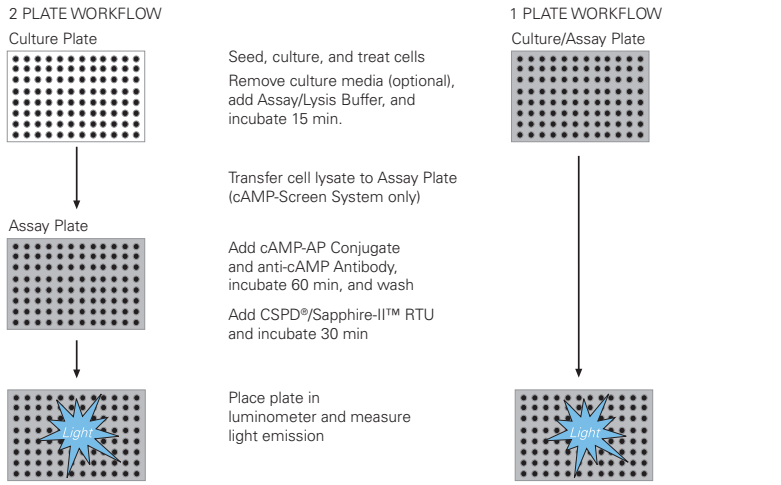
cAMP-Screen® and cAMP-Screen Direct® Systems

Chemiluminescent Immunoassay Systems for Determination of cAMP Concentration

- Rapid, convenient quantitation of intracellular cAMP
- High sensitivity and wide dynamic range
- Maximum chemiluminescence signal in 30 minutes, sustained for several hours
- Flexibility in assay format and growth surface
- Compatible with high-throughput screening applications
- No false positives—ideal for secondary screening



Panel A. cAMP-Screen® System Overview **Panel B. cAMP-Screen Direct® System Overview**

Figure 1. Overview of cAMP-Screen® and cAMP-Screen Direct® Systems. **Panel A.** cAMP-Screen System. Cells are cultured, treated, and lysed in tissue culture plates; cell lysates are then transferred to a solid white assay plate, pre-coated with secondary antibody. **Panel B.** cAMP-Screen Direct System. Cells are cultured, treated, lysed, and assayed in the assay plate. The cAMP-Screen Direct assay plates are tissue-culture treated, white microplates with clear bottoms, pre-coated with secondary antibody; clear-bottom wells permit visual inspection of cells prior to start of the assay.

The Tropix® cAMP-Screen® and cAMP-Screen Direct® Systems enable ultrasensitive determination of cAMP levels in cell lysates. These competitive immunoassay systems are formatted with maximum flexibility to permit either manual assay or automated high-throughput screening with 96- or 384-well microplates. Both systems utilize CSPD® substrate, a high-sensitivity chemiluminescent alkaline phosphatase (AP) substrate, and Sapphire-II™ luminescence enhancer. This ready-to-use substrate/enhancer reagent generates a light signal that is measured 30 minutes after addition, with glow emission persistence of several hours.

With the cAMP-Screen Direct System, cells are grown and lysed directly in the assay plate, eliminating the need for a separate culture plate. For cells requiring specialized growth surfaces, we recommend using the cAMP-Screen System after culturing cells in appropriate surface-modified culture plates.

Rapid Assay Formats

The rapid assay formats make the cAMP-Screen and cAMP-Screen Direct competitive immunoassay systems compatible with automated high-throughput screening instrumentation. Cells are seeded either into appropriate culture plates (cAMP-Screen System; Figure 1, Panel A) or into pre-coated assay plates (cAMP-Screen Direct System; Figure 1, Panel B), then cultured and treated with test compounds as desired.

With the cAMP-Screen System, cell lysates are prepared in the culture plate; culture media can be present or absent during lysis. A portion of each cell lysate is then transferred to the pre-coated assay plate. With the cAMP-Screen Direct System, cell lysates are prepared directly in the assay plate. The cAMP-Screen Direct System thus offers several advantages for high-throughput screening applications: reduced overall assay cost, because there is no requirement for a separate culture plate; a more streamlined protocol; and improved assay precision (%CV), because there is no transfer of cell lysate to an assay plate.

Once cell lysates are in the assay plate, the cAMP-Screen Direct and cAMP-Screen assays are identical. Lysates are incubated with a cAMP-AP conjugate and an anti-cAMP antibody in the secondary antibody-coated, luminometer-ready, assay plate; the resulting immune complexes are captured in the plate. The captured immune complexes are washed to remove unbound cAMP-AP, chemiluminescent substrate is added, and the resulting signal is measured in a luminometer without reagent injection (Figure 2).

A low level of intracellular cAMP gives minimal competition with the cAMP-AP conjugate for binding to anti-cAMP antibody, resulting in a high chemiluminescence signal. Increased levels of intracellular cAMP reduce the amount of cAMP-AP conjugate in the captured immune complexes. Standard curves display an inverse correlation between signal intensity and free cAMP concentration and allow quantitation of the amount of intracellular cAMP (Figures 3 and 4).

High Sensitivity and Wide Dynamic Range

The cAMP-Screen and cAMP-Screen Direct Systems provide sensitivity that is superior to any other commercially available cAMP assay: less than 0.2 pmol cAMP in the 384-well format. The dynamic range of both systems is outstanding: from less than 0.06 to greater than 6,000 pmol cAMP in the 96-well format (Figures 3A and 4), and from less than 0.2 to greater than 200 pmol cAMP in the 384-well format (Figure 3B), without sample dilution or modification (such as acetylation).

Quantitation of cellular cAMP levels is comparable in the cAMP-Screen and cAMP-Screen Direct Systems (Figure 5). Assay precision is very high for both systems; intra-assay precision for duplicate samples is typically 5% or less (data not shown). The substrate/enhancer must be allowed to reach the maximum glow signal to ensure an optimal coefficient of variance. In addition, the assay exhibits very low cross-reactivity with other adenosine-containing or cyclic nucleotides (Figure 6).

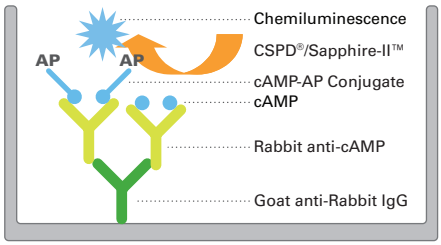
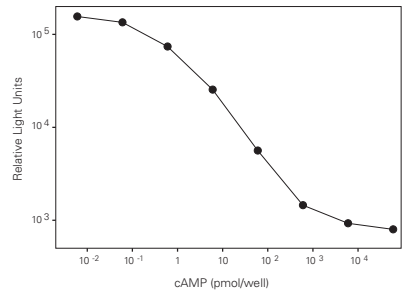
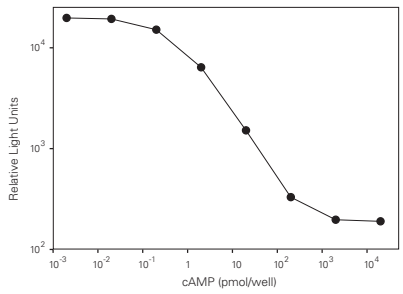


Figure 2. cAMP-Screen® and cAMP-Screen Direct® Immunoassay Systems: Competitive ELISA Format.



Panel A. 96-well Format



Panel B. 384-well Format

Figure 3. Dynamic Range of cAMP-Screen® System. The cAMP-Screen assay was performed in duplicate using cAMP standards in the indicated assay plate format. Signal was measured with the TR717™ microplate luminometer.

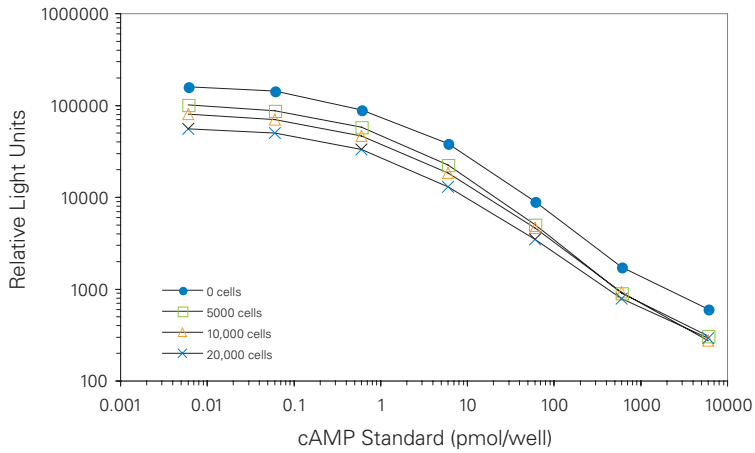


Figure 4. Sensitivity and Dynamic Range of the cAMP-Screen Direct® System. HEK293 cells were plated at the indicated densities and grown in cAMP-Screen Direct System 96-well assay plates, followed by duplicate cAMP-Screen Direct assays in the presence of cAMP standards. Sensitivity of the assay to exogenously added cAMP is unchanged following growth of cells in assay plate; signal intensity differences result from basal intracellular levels of cAMP. Signal was measured with the TR717™ microplate luminometer.

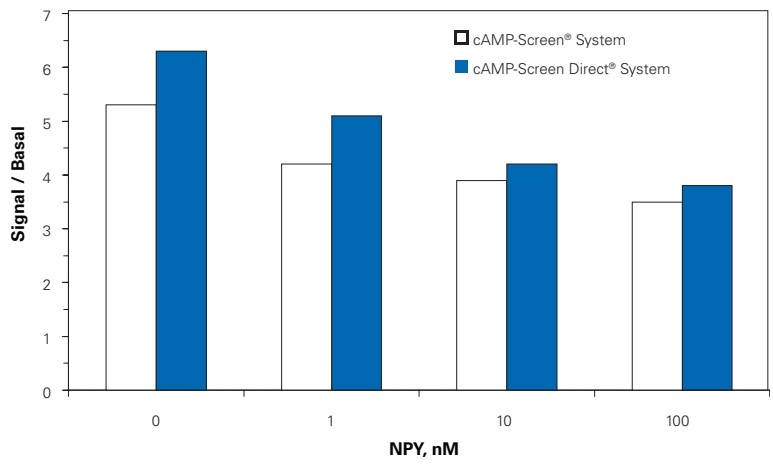


Figure 5. Comparison of the cAMP-Screen Direct® System with the cAMP-Screen® System for Quantitation of Intracellular cAMP Levels. SK-N-MC cells were cultured for four days in microplates (cAMP-Screen System) or in cAMP-Screen Direct System plates and then treated with 10 µM isoproterenol, to stimulate cAMP production, and NPY, an isoproterenol antagonist, at the indicated concentrations. For the cAMP-Screen System, cells were cultured in a separate culture plate, then lysates were prepared and transferred to a cAMP-Screen System plate and assayed in duplicate. For the cAMP-Screen Direct System, cell culture and assays (in duplicate) were performed in the same microplate.

Nucleotide	Cross-Reactivity (%)	Nucleotide	Cross-Reactivity (%)
cAMP	100	cGMP	0.02
AMP	0.15	cTMP	0.06
ADP	0.03	cUMP	0.012
ATP	0.15	cIMP	0.15

Figure 6. Cross-reactivity of cAMP-Screen® System with Related Nucleotides. Percent cross-reactivity is the ratio of the cAMP concentration to the cross-reactive nucleotide concentration that provides same assay signal intensity.

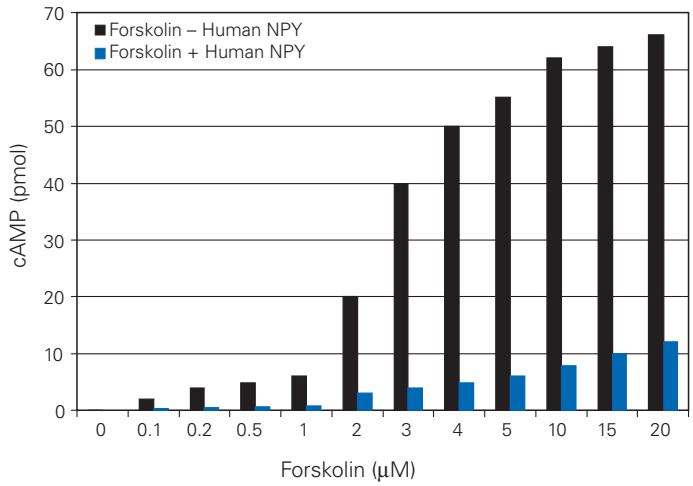


Figure 7. cAMP-Screen® Assay: Effect of Forskolin and an Antagonist on cAMP Levels. Duplicate cAMP-Screen assays were performed after using the indicated concentration of forskolin to induce cAMP production in SK-N-MC cells in the absence or presence of a forskolin antagonist (human NPY). Measurements were performed on a microplate luminometer.

Applications

The cAMP-Screen and cAMP-Screen Direct Systems are designed for rapid and convenient quantitation of cellular cAMP in functional assays of receptor activation, with both adherent and non-adherent cell types. These assays can be used with established cell lines for functional measurements with endogenous receptors (Figure 7), with cell lines with exogenously expressed ligand receptors, and with primary cell cultures in response to treatment of cells with the appropriate ligands. In addition, the cAMP-Screen and cAMP-Screen Direct Systems can be used for high-throughput screening assays for compounds which stimulate or inhibit cAMP production via interference with receptor function or signal transduction pathways. See the Literature/Resources tab at the Applied Biosystems web catalog page for the cAMP-Screen or cAMP-Screen Direct Systems for an applications bibliography.

Product Configurations

cAMP-Screen® and cAMP-Screen Direct® Immunoassay Systems are available in either 96-well or 384-well formats, and in sizes that are ideal for evaluation, research and development, or screening.

cAMP-Screen® Immunoassay System

Component	T1500	T1501	T1502	T1504
Plate Format	96-well	384-well	96-well	384-well
Assays per Kit	192	768	960	19,200
Assay/Lysis Buffer	25 mL	65 mL	2 x 65 mL	2 L
cAMP Standard	2 mL	5 mL	2 x 5 mL	100 mL
Anti-cAMP Antibody	14 mL	20 mL	2 x 35 mL	500 mL
cAMP-AP Conjugate	100 mL	250 mL	2 x 250 mL	5 mL
Conjugate Dilution Buffer	10 mL	25 mL	2 x 25 mL	500 mL
Wash Buffer	500 mL (1X)	1 L (1X)	2 x 1 L (1X)	5 x 1 L (5X)
CSPD®/Sapphire-II™ RTU	25 mL	25 mL	2 x 65 mL	650 mL
Pre-coated Plates	2 plates	2 plates	10 plates	50 plates

cAMP-Screen Direct® Immunoassay System

Component	T1505	T1506	T1507	T1508
Plate Format	96-well	384-well	96-well	384-well
Assays per Kit	192	768	960	19,200
Assay/Lysis Buffer	25 mL	65 mL	2 x 65 mL	2 L
cAMP Standard	2 mL	5 mL	2 x 5 mL	100 mL
Anti-cAMP Antibody	14 mL	20 mL	2 x 35 mL	500 mL
cAMP-AP Conjugate	100 mL	250 mL	2 x 250 mL	5 mL
Conjugate Dilution Buffer	10 mL	25 mL	2 x 25 mL	500 mL
Wash Buffer	500 mL (1X)	1 L (1X)	2 x 1 L (1X)	5 x 1 L (5X)
CSPD®/Sapphire-II™ RTU	25 mL	25 mL	2 x 65 mL	650 mL
Pre-coated Plates	2 plates	2 plates	10 plates	50 plates

ORDERING INFORMATION

Description	Size	P/N
cAMP-Screen® 96-Well Immunoassay System	192 assays	T1500
cAMP-Screen® 384-Well Immunoassay System	768 assays	T1501
cAMP-Screen® 96-Well Immunoassay System	960 assays	T1502
cAMP-Screen® 384-Well Immunoassay System	19,200 assays	T1504
cAMP-Screen Direct® 96-Well Immunoassay System	192 assays	T1505
cAMP-Screen Direct® 384-Well Immunoassay System	768 assays	T1506
cAMP-Screen Direct® 96-Well Immunoassay System	960 assays	T1507
cAMP-Screen Direct® 384-Well Immunoassay System	19,200 assays	T1508
Assay/Lysis Buffer	65 mL	T2327
1X Wash Buffer	1 L	T2337
5X Wash Buffer	1 L	T2356

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