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cAMP-Screen® System

cAMP-Screen Direct® System

Chemiluminescent Immunoassay System for the Quantitation of cAMP from Cultured Mammalian Cells in 96- or 384-Well Microplates

cAMP-Screen System P/N 4412182 (prev. T1500), 4412183 (prev. T1502), T1501, T1504

cAMP-Screen Direct System P/N 4412186 (prev. T1505), 4412187 (prev. T1507), T1506, T1508

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Part Number T9022 Revision D **Revision Date: November 2008**

For Research Use Only. Not for use in diagnostic procedures.

Information in this document is subject to change without notice.

Literature Citation: When describing a procedure for publication using these products, please refer to them as the cAMP-Screen® System or cAMP-Screen Direct® System, as appropriate.

PREFACE

Safety Information

Note: For general safety information, see this Preface and Appendix D, “Safety” on page 16. When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see the “Safety” Appendix for the complete alert on the chemical or instrument.

Safety Alert Words

Four safety alert words appear in Applied Biosystems user documentation at point in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

CAUTION! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

MSDSs

The MSDSs for any chemicals supplied by Applied Biosystems are available to you free 24 hours a day. For instructions on obtaining MSDSs, see MSDSs on page 17.

IMPORTANT! For the MSDSs of chemicals not distributed by Applied Biosystems contact the chemical manufacturer.

How to Obtain Support

For the latest services and support information for all locations, go to:

www.appliedbiosystems.com

At the Applied Biosystems web site, you can:

- Access worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents.
- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.

I. INTRODUCTION

The cAMP-Screen® and cAMP-Screen® Direct chemiluminescent ELISA systems are designed for the rapid and sensitive quantitation of 3',5'-cyclic AMP (cAMP) in extracts prepared from mammalian cells cultured in microwell plates without the need for sample acetylation or standards (1). The cAMP-Screen ELISA systems are a competitive immunoassay format that incorporates an alkaline phosphatase (AP)-labeled cAMP conjugate, a highly specific anti-cAMP antibody, precoated microplates, cAMP standard and CSPD® substrate with Sapphire-II™ luminescence enhancer to generate glow light emission kinetics. Light signal intensity is inversely proportional to the cAMP level in the sample or standard preparation, and is measured in a luminometer 30 min after substrate addition. The simple assay format and glow light emission kinetics achieved with cAMP-Screen reagents provide an ideal assay system for automated high-throughput screening applications. The kits include all required reagents and precoated microplates. The cAMP-Screen Direct enables cell culture directly in the pre-coated assay capture plate, for cell types not requiring a specialized growth surface, thus reducing microplate usage, eliminating the need for plate transfer step and reducing assay variability.

Cyclic AMP (cAMP) is an important second messenger in many signal transduction pathways linking activation of cell surface membrane receptors to intracellular responses, and ultimately, to changes in gene expression. cAMP is synthesized by plasma membrane-bound adenylate cyclase, which is coupled to transmembrane receptors for numerous hormones, neurotransmitters and other signaling molecules by heterotrimeric G-proteins. Upon ligand binding to GPCRs, the intracellular receptor domain interacts with a G protein, which then dissociates and activates adenylate cyclase activity, resulting in an increase in intracellular [cAMP]. Subsequently, cAMP activates cAMP-dependent protein kinases (protein kinase A), which phosphorylate specific substrate proteins including enzymes, structural proteins, transcription factors, ion channels, etc. In addition, both hormone-mediated activation and inhibition of adenylate cyclase has been demonstrated.

Chemiluminescent 1,2-dioxetane enzyme substrates provide detection sensitivity advantages in a wide variety of assay systems. 1,2-Dioxetane substrates provide an ultrasensitive assay with a wide dynamic assay range of cAMP quantitation. The cAMP-Screen ELISA systems provide a chemiluminescent assay solution for cAMP quantitation that is highly sensitive, simple to use, and amenable for use in automated, high-throughput screening applications.

Applications

The cAMP-Screen assay systems are designed for quantitation of cellular cAMP for functional assays of receptor activation. cAMP-Screen or cAMP-Screen Direct has been used with established cell lines for functional measurements with endogenous receptors (1-5), cell lines with exogenously expressed ligand receptors (6-8), primary cells (9-11), and tissues (12,13) in response to treatment with the appropriate ligands. The cAMP-Screen assay system has been used for receptor characterization (14,15), orphan receptor ligand identification (16), and the characterization of novel chimeric receptors (17). In addition, cAMP-Screen assay system can be used for high throughput screening assays (1,18) for compounds which stimulate or interfere with these signal transduction pathways.

II. SYSTEM COMPONENTS

Shelf-life for all kit components is six months when stored at 4°C.

cAMP-Screen System

	4412182 (prev. T1500) 96-Well	**4412183 (prev. T1502)** 96-Well	**T1501** 384-Well	**T1504** 384-Well
Microplate assays per kit	192	960	768	19,2000
Pre-coated Microplates (solid white plates)	2 plates	10 plates	2 plates	50 plates
Assay/Lysis Buffer	25 mL	2 x 65 mL	65 mL	2 L
cAMP Standard	2 mL	2 x 5 mL	5 mL	100 mL
Anti-cAMP Antibody	14 mL	2 x 35 mL	20 mL	500 mL
cAMP-AP Conjugate	100 µL	2 x 250 µL	250 µL	5 mL
Conjugate Dilution Buffer	10 mL	2 x 25 mL	25 mL	500 mL
Wash Buffer	500 mL (1X)	2 L (1X)	1 L (1X)	5 L (5X)
CSPD/Sapphire-II RTU Substrate/Enhancer Solution	25 mL	2 x 65 mL	25 mL	650 mL

cAMP-Screen Direct System

	4412186 (prev. T1505) 96-Well	**4412187 (prev. T1507)** 96-Well	**T1506** 384-Well	**T1508** 384-Well
Microplate assays per kit	192	960	768	19,200
Pre-coated Microplates (tissue culture-treated white with clear bottoms)	2 plates	10 plates	2 plates	50 plates
Assay/Lysis Buffer	25 mL	2 x 65 mL	65 mL	2 L
cAMP Standard	2 mL	2 x 5 mL	5 mL	100 mL
Anti-cAMP Antibody	14 mL	2 x 35 mL	20 mL	500 mL
cAMP-AP Conjugate	100 µL	2 x 250 µL	250 µL	5 mL
Conjugate Dilution Buffer	10 mL	2 x 25 mL	25 mL	500 mL
Wash Buffer	500 mL (1X)	2 L (1X)	1 L (1X)	5 L (5X)*
CSPD/Sapphire-II RTU Substrate/Enhancer Solution	25 mL	2 x 65 mL	25 mL	650 mL
Plate Backing Sheets	2 sheets	10 sheets	2 sheets	50 sheets

*5X Wash Buffer should be diluted in ultra-pure sterile ddH_2O.

Some additional components are available separately if more is required for automated plate washing systems.

Component	**P/N**	**Volume**
Assay/Lysis Buffer	T2327	65 mL
1X Wash Buffer	T2337	1 L
5X Wash Buffer	T2356	1 L

III. 96-WELL ASSAY PROTOCOL

Please read the entire Protocol and Notes sections before proceeding. For optimal performance, use the reagents supplied with the kit; the use of buffers not expressly approved by Applied Biosystems may adversely affect assay performance. All incubation steps are performed at room temperature unless noted otherwise.

A. Standard Preparation

The cAMP Standard supplied is 1 mM cAMP (1,000 pmol/µL). Prepare 7 serial 1:10 dilutions in Assay/Lysis buffer; this results in a concentration range from 0.006 to 6,000 pmol cAMP per 60 µL for the standard curve. Dilute Standard just prior to use.

For the following hazards, see the complete safety alert descriptions in Appendix D "Safety" on page 16:

⚠ **WARNING! CHEMICAL HAZARDS. Assay/Lysis Buffer.**

Dilution	cAMP, pmol/60 µL	cAMP-Standard	Assay/Lysis Buffer
1	6,000	30 µL cAMP Standard	270 µL
2	600	30 µL Dilution 1	270 µL
3	60	30 µL Dilution 2	270 µL
4	6	30 µL Dilution 3	270 µL
5	0.6	30 µL Dilution 4	270 µL
6	0.06	30 µL Dilution 5	270 µL
7	0.006	30 µL Dilution 6	270 µL
Blank	0	0 µL	300 µL

B. Cell Culture and Sample Preparation

cAMP-Screen Assay/Lysis Buffer is optimized for use with either adherent or non-adherent mammalian cell lines. A wide range of cell densities can be used (typically 1,000 to 50,000 cells/well, depending on the cell type), but this should be optimized to determine the cell density that provides the optimal experimental response. For the cAMP-Screen system, cells are cultured and experiment is performed in desired tissue culture-treated microplates (not provided). After sample preparation, cell lysates are transferred to the provided pre-coated assay plate. For the cAMP-Screen Direct system, cells are cultured directly in the pre-coated assay plate provided (tissue-culture treated and pre-coated with capture antibody). Sample preparation and cAMP quantitation assay are performed directly in the culture plate. For cells that require a specialized growth surface, the desired plates should be used for cell culture, and then the cAMP-Screen assay and plates are used for cAMP quantitation.

cAMP-Screen Assay

For the following hazards, see the complete safety alert descriptions in Appendix D "Safety" on page 16:

WARNING! CHEMICAL HAZARDS. Assay/Lysis Buffer.

1. Lysates may be prepared in either the presence or absence of culture media.

2. Following cell culture and experimental treatment (IBMX can be added to media as in cAMP-Screen Direct Step 1 below) in standard cell culture microplates, add a volume of Assay/Lysis Buffer (i.e., 100 µL) equivalent to the amount of culture medium used (i.e., for 100 µL of medium) to each well and mix. Or, remove cell culture media (adherent cells) and add 100 µL/well of Assay/Lysis Buffer.

3. Incubate at 37°C for 30 min or until cells are lysed (determined by microscopic observation).

cAMP-Screen Direct Assay

Adherent Cells

For the following hazards, see the complete safety alert descriptions in Appendix D "Safety" on page 16:

⚠ **WARNING! CHEMICAL HAZARDS. Assay/Lysis Buffer.**

1. Plate cells at desired density in cAMP-Screen Direct pre-coated assay plate, leaving necessary wells empty to perform desired standard curve. Typically, cells are seeded in 100 µL/well for up to several days at the appropriate growth conditions to reach the desired density. Next, if desired, remove media and replace with serum-free media (ex. 90 µL/well) containing IBMX (3-isobutyl-1-methylxanthone)phosphodiesterase inhibitor at 0.1-1mM final concentration. Then, add inducer/compound (ex. 5 µL/well) for the appropriate response time.

2. Remove media from wells and add 60 µL of Assay/Lysis Buffer.

3. Incubate at 37°C for 5-30 min, until cells are lysed (determined by microscopic observation).

Suspension Cells

For the following hazards, see the complete safety alert descriptions in Appendix D "Safety" on page 16:

⚠ **WARNING! CHEMICAL HAZARDS. Assay/Lysis Buffer.**

1. Plate cells at desired density in cAMP-Screen Direct pre-coated assay plate, leaving necessary wells empty to perform desired standard curve. Cells should be seeded at 25 µL/well and grown to desired density. Then add inducer/compound (ex. 5 µL/well) for the appropriate response time.

2. Add an equal volume (ie., 30 µL) of Assay/Lysis Buffer to wells containing cells.

3. Incubate at 37°C for 5-30 min, until cells are lysed (determined by microscopic observation).

C. Assay Procedure

For the following hazards, see the complete safety alert descriptions in Appendix D “Safety” on page 16:

WARNING! CHEMICAL HAZARDS. Anti-cAMP Antibody, CSPD®/Sapphire-II™ RTU Substrate/Enhancer Solution, Wash Buffer.

1. Dilute the cAMP-AP Conjugate 1:100 with Conjugate Dilution Buffer. Prepare 4 mL of diluted conjugate (40 µL cAMP-AP Conjugate + 3.96 mL of Conjugate Dilution Buffer) per 96-well plate. Dilute only sufficient conjugate for immediate use.

2. **cAMP-Screen assay**: Add 60 µL/well of cAMP Standard dilutions (or samples) and 30 µL/well of diluted cAMP-AP to wells of a pre-coated assay plate (provided), and mix by repeated pipetting or on a plate shaker.

   cAMP-Screen Direct assay: Add 60 µL/well of cAMP Standard dilutions to designated wells that have been left empty and 30 µL/well of diluted cAMP-AP to the entire assay plate, and mix by repeated pipetting or on a plate shaker.

3. Add 60 µL/well of anti-cAMP Antibody and mix by repeated pipetting or on a plate shaker.

4. Incubate for 1 hr. This assay was designed for use in automated robotic systems without shaking. However, results may improve with shaking.

5. Remove solution from wells and wash 6X with Wash Buffer.

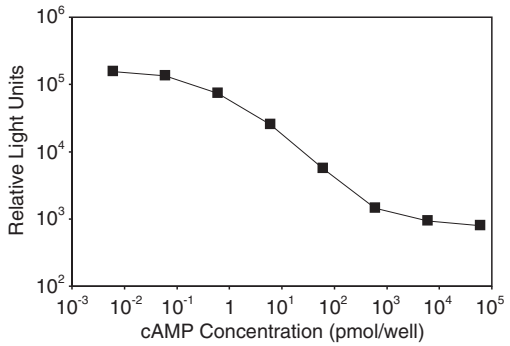
6. Add 100 µL/well of CSPD®/Sapphire-II™ RTU substrate/enhancer solution and incubate for 30 min. Measure signal in a luminometer for 1 sec/well or as appropriate for the instrument used.

   cAMP-Screen Direct assay: For optimal measurements on top reading luminometer, carefully apply the plate backing sheet to the bottom of microplate prior to reading.

D. Results

The cAMP-Screen (and cAMP-Screen Direct) assay is a competitive ELISA, so there is an inverse correlation between cAMP concentration in the sample and the assay signal intensity. Low levels of cAMP result in a high luminescence intensity, while a high concentration of cAMP results in a low signal. A sigmoidal standard curve is obtained, which is best fit with a weighted four-parameter logistic curve. An example of the standard curve obtained is shown below:

STANDARD CURVE



cAMP-Screen 96-well assays were performed with the cAMP Standard as described above. Measurements were made on the TR717™ microplate luminometer.

IV. 384-WELL ASSAY PROTOCOL

Please read the entire Protocol and Notes sections before proceeding. For optimal performance, use the reagents supplied with the kit; the use of buffers not expressly approved by Applied Biosystems may adversely affect assay performance. All incubation steps are performed at room temperature unless noted otherwise.

A Standard Preparation

The cAMP Standard supplied is 1 mM cAMP (1,000 pmol/µL). Prepare 7 serial 1:10 dilutions in Assay/Lysis buffer; this results in a concentration range from 0.002 to 2,000 pmol cAMP per 20 µL for the standard curve. Dilute Standard just prior to use.

For the following hazards, see the complete safety alert descriptions in Appendix D "Safety" on page 16:

⚠ **WARNING! CHEMICAL HAZARDS. Assay/Lysis Buffer.**

Dilution	cAMP, pmol/20 µL	cAMP-Standard	Assay/Lysis Buffer
1	2,000	30 µL cAMP Standard	270 µL
2	200	30 µL Dilution 1	270 µL
3	20	30 µL Dilution 2	270 µL
4	2	30 µL Dilution 3	270 µL
5	0.2	30 µL Dilution 4	270 µL
6	0.02	30 µL Dilution 5	270 µL
7	0.002	30 µL Dilution 6	270 µL
Blank	0	0 µL	300 µL

B. Cell Culture and Sample Preparation

cAMP-Screen Assay/Lysis Buffer is optimized for use with either adherent or non-adherent mammalian cell lines. A wide range of cell densities can be used (typically 500 to 25,000 cells/well, depending on the cell type), but this should be optimized to determine the cell density that provides the optimal experimental response. For the cAMP-Screen system, cells are cultured and experiment is performed in desired tissue culture-treated microplates (not provided). After sample preparation, cell lysates are transferred to the provided pre-coated assay plate. For the cAMP-Screen Direct system, cells are cultured directly in the pre-coated assay plate provided (tissue-culture treated and pre-coated with capture antibody). Sample preparation and cAMP quantitation assay are performed directly in the culture plate. For cells that require a specialized growth surface, the desired plates should be used for cell culture, and then the cAMP-Screen assay and plates are used for cAMP quantitation.

cAMP-Screen Assay

For the following hazards, see the complete safety alert descriptions in Appendix D "Safety" on page 16:

WARNING! CHEMICAL HAZARDS. Assay/Lysis Buffer.

1. Lysates may be prepared in either the presence or absence of culture media.

2. Following cell culture and experimental treatment (IBMX can be added to media as in cAMP-Screen Direct Step 1 below) in standard cell culture microplates, add a volume of Assay/Lysis Buffer (i.e., 25 µL) equivalent to the amount of culture medium used (i.e., for 25 µL of medium) to each well and mix. Or, remove cell culture media (adherent cells) and add 25 µL/well of Assay/Lysis Buffer.

3. Incubate at 37°C for 30 min or until cells are lysed (determined by microscopic observation).

cAMP-Screen Direct Assay

Adherent Cells

For the following hazards, see the complete safety alert descriptions in Appendix D “Safety” on page 16:

⚠ **WARNING! CHEMICAL HAZARDS. Assay/Lysis Buffer.**

1. Plate cells at desired density in cAMP-Screen Direct pre-coated assay plate, leaving necessary wells empty to perform desired standard curve. Typically, cells are seeded in 30 µL/well for up to several days at the appropriate growth conditions to reach the desired density. Next, if desired, remove media and replace with serum-free media (ex. 25 µL/well) containing IBMX (3-isobutyl-1-methylxanthone) phosphodiesterase inhibitor at 0.1-1mM final concentration. Then, add inducer/compound (ex. 2.5 µL/well) for the appropriate response time.

2. Remove media from wells and add 20 µL of Assay/Lysis Buffer.

3. Incubate at 37 °C for 5-30 min, until cells are lysed (determined by microscopic observation).

Suspension Cells

For the following hazards, see the complete safety alert descriptions in Appendix D “Safety” on page 16:

⚠ **WARNING! CHEMICAL HAZARDS. Assay/Lysis Buffer.**

1. Plate cells at desired density in cAMP-Screen Direct pre-coated assay plate, leaving necessary wells empty to perform desired standard curve. Typically, cells should be added at 8 µL/well and grown to desired density. Then add inducer/compound (ex. 1 µL/well) for the appropriate response time.

2. Add an equal volume (ie., 10 µL) of Assay/Lysis Buffer to wells containing cells.

3. Incubate at 37 °C for 5-30 min, until cells are lysed (determined by microscopic observation).

C. Assay Procedure

For the following hazards, see the complete safety alert descriptions in Appendix D “Safety” on page 16:

⚠ **WARNING! CHEMICAL HAZARDS. Anti-cAMP Antibody, CSPD®/Sapphire-II™ RTU Substrate/Enhancer Solution, Wash Buffer.**

1. Dilute the cAMP-AP Conjugate 1:100 with Conjugate Dilution Buffer. Prepare 4 mL of diluted conjugate (40 µL cAMP-AP Conjugate + 3.96 mL of Conjugate Dilution Buffer) per 96-well plate. Dilute only sufficient conjugate for immediate use.

2. **cAMP-Screen assay**: Add 20 µL/well of cAMP Standard dilutions (or samples) and 10 µL/well of diluted cAMP-AP to wells of a pre-coated assay plate (provided), and mix by repeated pipetting or on a plate shaker.

   cAMP-Screen Direct assay: Add 20 µL/well of cAMP Standard dilutions to designated wells that have been left empty and 10 µL/well of diluted cAMP-AP to the entire assay plate, and mix by repeated pipetting or on a plate shaker.

3. Add 20 µL/well of anti-cAMP Antibody and mix by repeated pipetting or on a plate shaker.

4. Incubate for 1 hr. This assay was designed for use in automated robotic systems without shaking. However, results may improve with shaking.

5. Remove solution from wells and wash 6X with Wash Buffer.

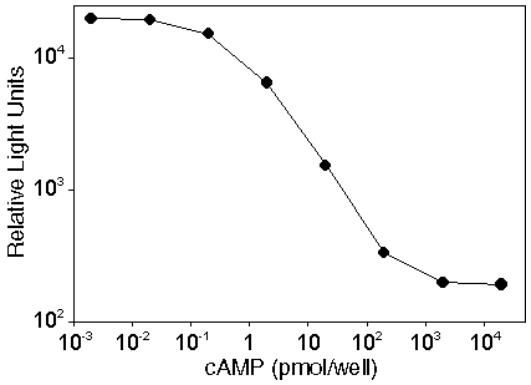
6. Add 30 µL/well of CSPD®/Sapphire-II™ RTU substrate/enhancer solution and incubate for 30 min. Measure signal in a luminometer for 1 sec/well or as appropriate for the instrument used.

   cAMP-Screen Direct assay: For optimal measurements on top reading luminometer, carefully apply the plate backing sheet to the bottom of microplate prior to reading.

D. Results

The cAMP-Screen (and cAMP-Screen Direct) assay is a competitive ELISA, so there is an inverse correlation between cAMP concentration in the sample and the assay signal intensity. Low levels of cAMP result in a high luminescence intensity, while a high concentration of cAMP results in a low signal. A sigmoidal standard curve is obtained, which is best fit with a weighted four-parameter logistic curve. An example of the standard curve obtained is shown below:

STANDARD CURVE



cAMP-Screen 384-well assays were performed with the cAMP Standard as described above. Measurements were made on the TR717™ microplate luminometer.

V. APPENDICES

A. Use of Luminometers

We recommend using a single-mode luminometer or a multi-mode detection instrument set for luminescence measurement to measure light emission from 96- or 384-well microplates. Contact Applied Biosystems Technical Support group for additional questions.

B. Cross Reactivity Specifications

cAMP	100%
cGMP	0.02%
cUMP	0.012%
cIMP	0.15%
cTMP	0.06%
CTP	0.002%
GMP	0.005%
GTP	0.2%
AMP	0.15%
ADP	0.03%
ATP	0.15%

C. Frequently Asked Questions (FAQs)

Question #1: Cloudy and Viscous Assay/Lysis Buffer
My Assay/Lysis Buffer is cloudy and/or viscous. Will this affect the performance of my cell lysis or assay?
Answer: The detergent in the Assay/Lysis buffer has a low cloud point of ~20 °C, where it becomes cloudy and separates into an aqueous and a detergent phase. Always make sure that the Assay/Lysis buffer is stored cold and is mixed before use, as the detergent will settle to the bottom.

Question #2: Proven Cell Types
Will my experimental cell line work with the cAMP-Screen assay systems?
Answer: A wide variety of cell types (tissue origin), as well as primary cells (platelets, adipocytes, hepatocytes, lymphocytes, osteoblasts, fibroblasts) and cell lines including cells with expressing endogenous ligand receptors (SK-N, AtT20/D, NCI-H716, T84, A549, THP-1) and transfected (exogenous) ligand receptors (CHO, HEK293) have been used successfully with the cAMP-Screen systems. The cAMP-Screen and cAMP-Screen Direct bibliographies (available on our web site) provide annotation with each reference that indicates the cell type being used for further information.

Question #3: Cell Lysis
When I use your suggested cell lysis incubation, I don't really see complete cell lysis when I observe cells under microscope. Are my cells really lysed? What should I do differently?
Answer: Firstly, as above, make sure that the Assay/Lysis Buffer is mixed well before use, as the detergent can settle to the bottom, to ensure the correct detergent concentration. It is requently observed that there is some remaining cell structure visible. Since cAMP is a small molecule, and is soluble, permeabilization of cells will enable release from cells. It is not necessary to observe "complete" lysis to the extent that cells are no longer visible to ensure adequate results. Incubation time can be increased, if desired.

Question #4: Modified Culture Surfaces
My cells require a specialized growth surface (poly-lysine-coated, collagen-coated tissue culture plates). Which kit should I use? Can I use cAMP-Screen Direct?
Answer: For cells requiring a specialized growth surface, you should use the required plates for cell culture, prepare cell lysate in those plates, and then use the cAMP-Screen system for cAMP quantitation. The cAMP-Screen Direct pre-coated microplates (antibody pre-coated) should not be coated with anything else, and will probably not provide the correct growth surface. So, for these types of cells, the cAMP-Screen system should be used, not the cAMP-Screen Direct system.

Question #5: Plate Edge Effects
I am seeing edge effects on my assay plates, where signals in outer wells of the plate have lower or higher signal levels than middle wells. What can I do to minimize these effects?
Answer: Edge effects can be caused by uneven evaporation of the reagents, or temperature fluctuation of the plate platform in instrument. Cover or seal the plate during incubations. Make sure that your luminometer is warmed up before reading.

Question #6: Plate Read Time
When can I read my assay plates?
Answer: The assay plates can be read at 30 minutes. If multiple plates are being run together and compared to each other, take care to determine the optimal time after substrate addition to read the plates. Peak RLU in some cases may not be reached until 45 to 60 minutes after substrate addition, and will be maintained for several hours. When comparing results from multiple plates we advise reading plates once they have reached the peak RLU.

Question #7: Partial Plate Use
Can I use only a portion of the assay plate now and save the remaining wells for use at a later time?
Answer: We do not recommend doing this for concern of contamination of unused wells.

Question #8: cAMP-Screen system vs. cAMP-Screen Direct system
What is the difference between the cAMP-Screen and cAMP-Screen Direct kits?
Answer: The only difference is that the Direct version has assay plates that are tissue culture-treated with clear-bottom wells. This enables cell culture and visualization directly in the assay plate, and eliminates use of a separate culture plate and transfer of cell lysate from the culture plate to the assay plate. If your cells require special tissue culture plates to grow (ex. poly-D-lysine-coated plates), we advise using the cAMP-Screen kit.

Question #9: Luminometer Instrumentation
What luminometer do yourecommend for reading cAMP-Screen and cAMP-Screen Direct assays?
Answer: The light signal obtained with these assay systems exhibits "glow" light emission kinetics, and has a very broad emission spectrum, with an emission maximum of ~470 nm. You can use either dedicated (single-mode) luminometers or multi-mode platforms, set to read in the luminescence mode. Reagent injectors are not required. If instrument is a multi-mode instrument, there should be no excitation or emission filters in place, as the use of filters can possibly block some of the light signal and reduce sensitivity. Our customers have used a wide variety of luminometer instrumentation, including the Microlumat (Berthold Technologies), Wallac 1420 Victor (Perkin-Elmer), Packard Topcount NXT (Perkin-Elmer), BMG POLARstar Galaxy (BMG Labtech) and the Luminoskan Ascent (Thermo Labsystems). There are really no special instrumentation requirements for measurement of the cAMP-Screen assays, other than using a good quality microplate luminometer. You cannot use a microplate fluorometer or spectrophotometer to measure chemiluminescence (unless there is a luminometer reading mode on the instrument).

D. Safety

General chemical safety

Chemical hazard warning

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Material Safety Data Sheet (MSDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING! CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See “About MSDSs” on page 17.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer’s cleanup procedures as recommended in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

MSDSs

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to new customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

The MSDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain MSDSs:

1. Go to www.appliedbiosystems.com, click **Support**, then select **MSDS**.

2. In the Keyword Search field, enter the chemical name, product name, MSDS part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click Search.

3. Find the document of interest, right-click the document title, then select any of the following:
    - **Open** – To view the document
    - **Print Target** – To print the document
    - **Save Target As** – To download a PDF version of the document to a destination that you choose

Note: For the MSDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical waste safety

Chemical waste hazards

CAUTION! HAZARDOUS WASTE. Refer to Material Safety Data Sheets and local regulations for handling and disposal.

WARNING! CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

  IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

General biohazard

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories (stock no. 017-040-00547-4; bmbl.od.nih.gov)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

IMPORTANT! Additional information about biohazard guidelines is available at: www.cdc.gov

Chemical alerts

For the definitions of the alert words **IMPORTANT**, **CAUTION**, **WARNING**, and **DANGER**, see "Safety alert words" on page 1.

General alerts for all chemicals

EXAMPLE: Avoid contact with (skin, eyes, and/or clothing). Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Specific chemical alerts

WARNING! CHEMICAL HAZARD. Anti-cAMP Antibody may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING! CHEMICAL HAZARD. Assay/Lysis Buffer may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING! CHEMICAL HAZARD. CSPD®/Sapphire-II™ RTU Substrate/Enhancer Solution may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING! CHEMICAL HAZARD. Wash Buffer causes eye, skin, and respiratory tract irritation. Avoid breathing vapor. Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

VI. REFERENCES

cAMP-Screen® Immunoassay System

1. Chiulli, AC, K Trompeter and M Palmer (2000). A novel high throughput chemiluminescent assay for the measurement of cellular cyclic adenosine monophosphate levels. *J Biomol Screen* 5(4):239-247. (SK-N-MC human neuroblastoma cell line)
2. Hoare, SRJ, B Fleck, RS Gross, PD Crowe, JP Williams, and DE Grigoriadis (2008). Allosteric ligands for the corticotrophin releasing factor type 1 receptor modulate conformational states involved in receptor activation. *Molecular Pharmacology* 73(5):1371-1380.(CHO cells expressing human CRF_1 receptors and mouse AtT20/D 16v-F2 corticotrope cells expressing endogenous CRF_1 receptors)
3. Sato, H, A Macchiarulo, C Thomas, A Gioiello, M Une, AF Hormann, R Saladin, K Schoonjans, R Pellicciari and J Auwerx (2008). Novel potent and selective bile acid derivatives as TGR5 agonists: Biological screening, structure-activity relationships, and molecular binding studies. *J Med Chem* 51:1831-1841. (CHO cells overexpressing exogenous TGR5, NCI-H716 intestinal enteroendocrine cell line endogenously epxressing TGR5)
4. Kolachala, V, V Asamoah, L Wang, S Srinivasan, D Merlin and SV Sitaraman (2005). Interferon-γ down-regulates adenosine 2b receptor-mediated signaling and short circuit current in the intestinal epithelia by inhibiting the expression of adenylate cyclase. *J Biol Chem* 280(6):4048-4057. (colonic epithelia T84 cells)
5. Hong, T, AJ Kastin and W Pan (2007). Corticotropin-releasing hormone receptor (CRHR)1 and CRHR2 are both trafficking and signaling receptors for urocortin. *Molecular Endocrinology (USA)* 21(3):700-711. (transfected HK293 cells)
6. Koike, D, H Obinata, A Yamamoto, S Takeda, H Komori, F Nara, T Izumi and T Haga (2006). 5-Oxo-eicosatetraenoic acid-induced chemotaxis: Identification of a responsible receptor hGPCR48 and negative regulation by G protein $G_{12/13}$. *J Biochem* 139:543-549. (stably-transfected CHO cells)
7. Lappas, CM, JM Rieger and J Linden (2005). A_{2A} adenosine receptor induction inhibits IFN-γ production in murine $CD4^+$ T cells. *J Immunol* 174(1):1073-1080. (primary murine $CD4^+$ T cells)
8. NiiKono, T, Y Iwasaki, M Uchida, A Fujieda, A Hosokawa, M Motojima, H Yamato, K Kurokawa and M Fukagawa (2007). Indoxyl sulfate induces skeletal resistance to parathyroid hormone in cultured osteoblastic cells. *Kidney International* 71(8):738-743. (primary mouse osteoblastic cells)
9. Andre, P, SM Delaney, T LaRocca, D Vincent, F DeGuzman, M Jurek, B Koller, DR Phillips and PB Conley (2003). $P2Y_{12}$ regulates platelet adhesion/activation, thrombus growth, and thrombus stability in injured arteries. *J Clin Invest* 112(3):398-406. (murine platelets)
10. Fatholahi, M, V Xiang, Y Wu, Y Li, L Wu, AK Dhalla, L Belardinelli and JC Shryock (2006). A novel partial agonist of the A1-adenosine receptor and evidence for receptor homogeneity in adipocytes. *J Pharmacol Exp Therapeutics* 317(2):676-684. (rat adipocytes)
11. Inbe, H, S Watanabe, M Miyawaki, E Tanabe and JA Encinas (2004). Identification and characterization of a cell-surface receptor, P2Y15, for AMP and adenosine. *J Biol Chem* 279(19):19790-19799. (stably-transfected HEK293)
12. Nickolls, SA, MI Cismowski, X Wang, M Wolff, PJ Conlon and RA Maki (2003). Molecular determinants of melanocortin 4 receptor ligand binding and MC4/MC3 receptor selectivity. *J Pharmacol Exp Therapeutics* 304(3):1217-1227. (stably-transfected HEK293)
13. He, W, FJ-P Miao, DC-H Lin, RT Schwandner, Z Wang, J Gao, J-L Chen, H Tian and L Ling (2004). Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. *Nature* 429:188-193. (transiently-transfected CHO)
14. Gupte, J, G Cutler, J-L Chen and H Tian (2004). Elucidation of signaling properties of vasopressin receptor-related receptor 1 by using the chimeric receptor approach. *Proc Nat Acad Sci USA* 101(6):1508-1513. (stably- and transiently-transfected HEK293)
15. Vater, A, F Jarosch, K Buchner and S Klussmann (2003). Short bioactive Spiegelmers to migraine-associated calcitonin gene-related peptide rapidly identified by a novel approach: Tailored-SELEX. *Nuc Acid Res* 31(21):e130. (SK-N-MC human neuroblastoma cells)

cAMP-Screen Direct® Immunoassay Systems

1. McKenna, SD, G Feger, C Kelton, M Yang, V Ardissone, R Cirillo, P-A Vitte, X Jiang and RK Campbell (2007). Tumor necrosis factor (TNF)-soluble high affinity receptor complex as a TNF antagonist. *J Pharmacol Exp Therapeutics* 322(2):822-828. (human A549 lung carcinoma cells)
2. Garczynski, SF, JW Crim and MR Brown (2007). Characterization and expression of the short neuropeptide F receptor in the african malaria mosquito, *Anopheles gambiae*. Peptides 28(1):109-118. (CHO expressing Ang-sNPFR)
3. Zhong, H, Y Wu, L Belardinelli and D Zhen (2006). A_{2B} adenosine receptors induce IL-19 from bronchial epithelial cells and results in TNF-alpha increase. *Am J Respir Cell Mol Biol* 35(5):587-592. (primary human bronchial epithelial cells (HBECs), THP-1 human monocytic cells)

For complete, updated reference lists, please see http://www.appliedbiosystems.com (Product & Service Literature).