cAMP/PKA Signaling Pathway
CRE/CREB Reporter (Luc) – HEK293 Cell Line

#60515

Description

The cAMP/PKA Signaling Pathway CRE/CREB Reporter (Luc) – HEK293 Cell Line contains a firefly luciferase gene under the control of multimerized cAMP response element (CRE) stably integrated into HEK293 cells. Elevation of the intracellular cAMP level activates cAMP response element binding protein (CREB) to bind CRE and induces the expression of luciferase. This cell line is validated for response to stimulation by forskolin and to the treatment with an inhibitor of the cAMP/PKA signaling pathway.

Background

The cAMP/PKA Signaling Pathway CRE/CREB Reporter (Luc) – HEK293 Cell Line is designed for monitoring the activity of the cAMP/ PKA signaling pathway. The main role of the cAMP response element, or CRE, is mediating the effects of Protein Kinase A (PKA) by way of transcription. It is the main binding site of cAMP response element binding protein (CREB) and is responsible for its activation. CRE is the target of many extracellular and intracellular signaling pathways, including cAMP, calcium, GPCR (G-protein coupled receptors) and neurotrophins. The cAMP/PKA signaling pathway is critical to numerous life processes in living organisms. In the cAMP/PKA signaling pathway, CREB is activated via phosphorylation of PKA and binds to CRE with a general motif of 5'-TGACGTCA-3'. Since CRE is a modulator of the cAMP/PKA signaling pathway, it allows the effects of various inhibitors to be studied.

Application

- Monitor cAMP/PKA signaling pathway activity.
- Screen for compound activity of the cAMP/PKA signaling pathway.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains ~1.5 x 10^6 cells in 1 ml of 10% DMSO

Host Cell

HEK293

Mycoplasma Testing

The cell line has been screened using the PCR-based Venor™GeM Mycoplasma Detection kit (Sigma-Aldrich) to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with this cell line but are necessary for cell culture and cellular assays. BPS Bioscience reagents systems are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section.

Materials Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1F	BPS Bioscience #79540

BPS Bioscience
● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

Materials Required for Cellular Assay

Name	Ordering Information
Forskolin: 10 mM in DMSO: activator of cAMP expression	LC Laboratories #F-9929
H-89: 10 mM in DMSO: inhibitor of cAMP/PKA pathway	Enzo Life Sciences #BML-EI196-0005
Assay Medium: Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1F	BPS Bioscience #79540
96-well tissue culture treated white clear-bottom assay plate	Corning #3610
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Storage Conditions

Cells will arrive upon dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. To formulate a comparable but not BPS validated media, formulation components can be found below.

Note: Thaw Media does *not* contain selective antibiotics. However, Growth Media *does* contain selective antibiotics, which are used for maintaining cell lines over many passages. Cells should be grown at 37°C with 5% CO_2. BPS cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):
MEM medium (Thermo Fisher, #11095098) supplemented with 10% FBS (Thermo Fisher, #26140079), 1% non-essential amino acids (Corning, #25-025-CI), 1 mM Na pyruvate (Corning, #25-000-CI), 1% Penicillin/Streptomycin (Thermo Fisher, #15140163)

Growth Medium 1F (BPS Bioscience #79540):
Thaw Medium 1 (BPS Bioscience #60187) and 100 µg/ml of Hygromycin B (Hyclone #SV30070.01).

Assay Medium: Thaw Medium 1 (BPS Bioscience #60187)

Cell Culture Protocol

Cell Thawing

1. To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of Thaw Medium 1 (**no hygromycin**), spin down cells (1000 rpm), and resuspend cells in pre-warmed Thaw Medium 1 (**no hygromycin**).
2. Transfer resuspended cells to a single T25 flask and culture at 37°C in a 5% CO_2 incubator.
3. At first passage, switch to Growth Medium 1F (**contains hygromycin**).
4. Cells should be split before they reach complete confluence.

Cell Passage

1. To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with Trypsin/EDTA, add Growth Medium 1F and transfer to a tube.
2. Spin down cells, resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels.

Cell Freezing

1. To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with Trypsin/EDTA.
2. Add Growth Medium 1F and transfer to a tube, spin down cells, and resuspend in freezing medium (10% DMSO + 90% FBS).
3. Place at -80°C overnight in a cell freezing rack designed to cool the cells slowly and place in liquid nitrogen the next day.

Note: Just after thawing and at low density, the cells may grow at a slower rate. It is recommended to split the cells with ~ 1:4 ratio at the beginning of culturing. After several passages, the cell growth rate increases and the cells can be split with 1:8 -1:20 ratio weekly.

Note: It is recommended to expand the cells and freeze down at least 10 vials of cells at an early passage for future use.

Validation Data

The following assays are designed for 96-well format. To perform the assay in different tissue culture formats, cell number and reagent volumes should be scaled appropriately.

A. Dose response of *cAMP/ PKA Signaling Pathway CRE/CREB Reporter (Luc) – HEK293* cells to Forskolin

1. Harvest CRE/ CREB reporter (Luc)-HEK293 cells from culture in Growth Medium 1F and seed cells at a density of 30,000 cells per well in 75 µl of assay medium into a white clear-bottom 96-well microplate.
2. Incubate cells at 37°C in a CO_2 incubator overnight.
3. Add 25 µl of threefold serial dilution of forskolin in assay medium to stimulated wells.
   Add 25 µl of assay medium to the unstimulated control wells.
   Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).
   Set up each treatment in at least triplicate.
4. Incubate the plate at 37°C in a CO_2 incubator for 5-6 hours.
5. Perform luciferase assay using ONE-Step™ Luciferase Assay System according to the protocol provided:
   Add 100 µl of ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 minutes.
   Measure luminescence using a luminometer. If using other luciferase reagents from other vendors, follow the manufacturer's assay protocol.
6. Data Analysis: Subtract average background luminescence (cell-free control wells) from luminescence reading of all wells. The fold induction of CRE/CREB luciferase reporter expression = background-subtracted luminescence of forskolin-stimulated wells / average background-subtracted luminescence of unstimulated control wells

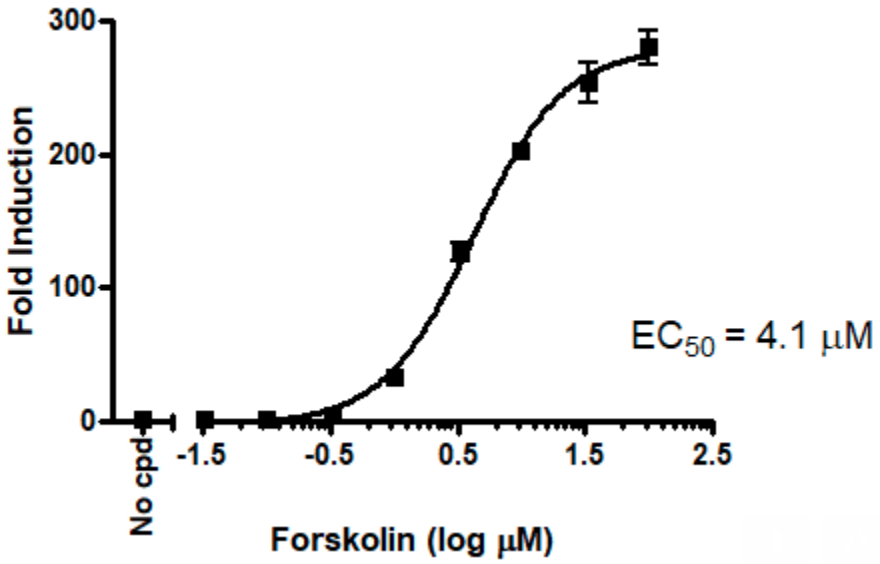

Figure 1. Dose response of CRE/CREB reporter (Luc)-HEK293 cells to forskolin. The results are shown as fold induction of CRE/CREB luciferase reporter expression.

B. Inhibition of forskolin-induced reporter activity by an inhibitor of cAMP/PKA signaling pathway

1. Harvest CRE/CREB reporter (Luc)-HEK293 cells from culture in Growth Medium 1F and seed cells at a density of 30,000 cells per well in 50 µl of assay medium into a white clear-bottom 96-well microplate.
2. Dilute the inhibitor (H-89 or other test compound) in assay medium at 2x the final concentration and add 50ul of compound dilution in assay medium to the wells. The final concentration of DMSO in assay medium can be up to 0.5%.
   Add 50 µl of assay medium with same concentration of DMSO without inhibitor to control wells.
   Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).
   Set up each treatment in at least triplicate.
   Incubate cells at 37°C in a CO_2 incubator overnight.
3. Add 10 µl of diluted forskolin in assay medium to stimulated wells (final [forskolin] = 10 µM).
   Add 10 µl of assay medium to the unstimulated control wells.
4. Incubate the plate at 37°C in a CO_2 incubator for 5-6 hours.
5. Perform luciferase assay using ONE-Step™ Luciferase Assay System according to the protocol provided:
   Add 110 µl of ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 minutes.
   Measure luminescence using a luminometer.
   If using other luciferase reagents from other vendors, follow the manufacturer's assay protocol.
6. Data Analysis: Obtain background-subtracted luminescence by subtracting average background luminescence (cell-free control wells) from luminescence reading of all wells.

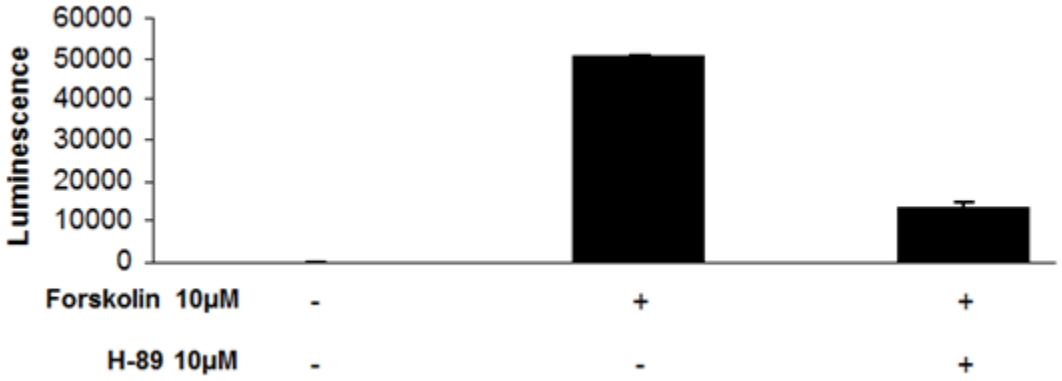

Figure 2. Inhibition of forskolin-induced reporter activity by H-89 in CRE/CREB reporter (Luc)-HEK293 cells

References:

1. Montminy, M.R. *et al.* (1987) Binding of a nuclear protein to the cyclic-AMP response element of the somatostatin gene. Nature **328(6126):** 175-178.
2. Fan Chung, K. (2006) Phosphodiesterase inhibitors in airways disease. Eur. J. Pharmacol. **533(1-3):** 110-117.
3. Malik, R. *et al.* (2008) Cloning, stable expression of human phosphodiesterase 7A and development of an assay for screening of PDE7 selective inhibitors. Appl. Microbiol. Biotechnol. **77 (5):** 1167-1173.

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
Growth Medium 1F	79540	500 ml
Thaw Medium 1	60187	Various Sizes
ONE-Step™ Luciferase Assay System	60690	Various Sizes
CRE/CREB Reporter Kit (cAMP/PKA Signaling Pathway)	60611	500 reactions