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

#60515

Description

The CRE/CREB Luciferase Reporter HEK293 Cell Line (cAMP/PKA Signaling Pathway) is an HEK293 cell line that contains a firefly luciferase reporter under the control of multimerized cAMP response element (CRE), designed to monitor the activity of the cAMP/ PKA signaling pathway. Elevation of intracellular cAMP levels activates cAMP response element binding protein (CREB) to bind to CRE and induces expression of luciferase. This cell line is validated for response to stimulation by forskolin and to treatment with an inhibitor of the cAMP/PKA signaling pathway.

Background

The cAMP/PKA signaling pathway is critical to numerous life processes in living organisms. 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. 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 on the cAMP/PKA signaling pathway.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains >1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Host Cell

HEK293, Human Embryonic Kidney, epithelial-like cells, adherent

Mycoplasma Testing

The cell line has been screened 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

Our products are for research use only, not for diagnostic or therapeutic use
BPS Bioscience
● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

Materials Required for Cellular Assay

Name	Ordering Information
Forskolin 10 mM in DMSO	LC Laboratories #F-9929
H-89: 10 mM in DMSO	Enzo Life Sciences #BML-EI196-0005
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 For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media does *not* contain selective antibiotics. However, Growth Media *does* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene of interest. Cells should be grown at 37°C with 5% CO_2. BPS cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Growth Medium 1F (BPS Bioscience #79540):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin plus 100 µg/ml Hygromycin B.

Media Required for Functional Cellular Assay

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

Cell Culture Protocol

Note: HEK293 cells are derived from human material and thus the use of adequate safety precautions is recommended.

Cell Thawing

1. Retrieve a cell vial from liquid nitrogen storage. Keep on dry ice until ready to thaw.

2. When ready to thaw, swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. Once cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire content of the vial to an empty 50 ml conical tube.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

3. Using a 10 ml serological pipette, slowly add 10 ml of pre-warmed Thaw Medium 1 to the conical tube containing the cells. Thaw Medium 1 should be added dropwise while gently rocking the conical tube to permit gentle mixing and avoid osmotic shock.

4. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 1.

5. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator.

6. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 and continue growing culture in a 5% CO_2 incubator at 37°C until the cells are ready to be split.

7. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 1F.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1F and transfer to a tube.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1F.

4. Seed into new culture vessels at an appropriate sub-cultivation ratio.

   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.

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1B and count the cells.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~2 x 10^6 cells/ml.

For research use only. Not for human, drug, diagnostic, or therapeutic use.
BPS Bioscience
● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

4. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

5. Transfer the vials to liquid nitrogen the next day for long term storage.

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

Functional Validation

- 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.
- The experiments should be performed in triplicate.
- The assay should include “Cell-Free Control”, “Unstimulated Control” and “Stimulated” conditions.

Assay Medium: Thaw Medium 1

A. Dose response of CRE/CREB Luciferase Reporter HEK293 Cell Line to Forskolin

1. Harvest CRE/CREB Luciferase Reporter 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. Leave empty wells to determine the background luminescence.

2. Incubate cells at 37°C in a CO_2 incubator overnight.

3. Prepare a threefold serial dilution of forskolin at concentrations 4-fold higher than the desired final concentration in Assay Medium (25 µl/well).

4. Add 25 µl diluted forskolin to the “Stimulated” wells.

5. Add 25 µl of Assay Medium to the “Unstimulated Control” wells.

6. Add 100 µl of Assay Medium to the “Cell-Free Control” wells (for determining background luminescence).

7. Incubate the plate at 37°C in a CO_2 incubator for 5-6 hours.

8. Add 100 µl of ONE-Step™ Luciferase reagent to each well.

9. Rock at Room Temperature (RT) for ~15 minutes.

10. Measure luminescence using a luminometer.

For research use only. Not for human, drug, diagnostic, or therapeutic use.
BPS Bioscience
• bpsbioscience.com • 858-202-1401 • support@bpsbioscience.com

11. Data Analysis: Subtract average background luminescence (cell-free control wells) from luminescence reading of all wells. The fold induction of CRE luciferase reporter expression is the average background-subtracted luminescence of the stimulated wells divided by the average background-subtracted luminescence of the unstimulated control wells.

$$Fold\ induction = \frac{(luminescence\ of\ stimulated\ cells - average\ background)}{(average\ luminescence\ of\ unstimulated\ cells - average\ background)}$$

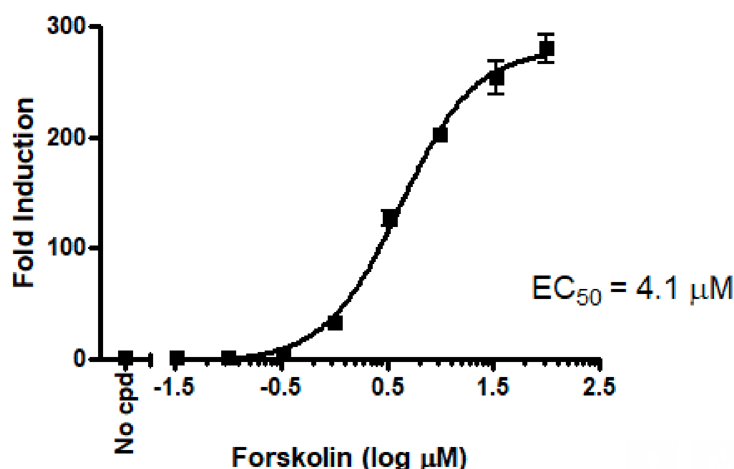

Figure 1. Dose response of CRE/CREB Luciferase Reporter HEK293 Cell Line to forskolin.
Cells were treated with increasing concentrations of forskolin in a 96-well plate. Luciferase activity was measured with ONE-Step™ Luciferase Assay System. Results are expressed as fold induction versus the unstimulated control. 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 Luciferase Reporter 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. Leave empty wells to determine the background luminescence.

2. Prepare the Test Compound, such as H-98, (50 µl/well): for a titration, prepare serial dilutions at concentrations 2-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   2.1. If the Test Compound is water-soluble, prepare serial dilutions in Assay Medium at concentrations 2-fold higher than the desired final concentrations.

   For the positive and negative controls, use Assay Medium (Diluent Solution).

   OR

For research use only. Not for human, drug, diagnostic, or therapeutic use.
● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com
BPS Bioscience

2.2. If the Test compound is soluble in DMSO, prepare the test compound in 100% DMSO at a concentration 200-fold higher than the highest desired final concentration, then dilute the inhibitor 100-fold in Assay Buffer to prepare the highest concentration of the serial dilutions. The concentration of DMSO is now 1%.

Using the Assay Medium containing 1% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Compound at 2-fold the desired final concentrations.

For positive and negative controls, prepare 1% DMSO in Assay Medium (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO in the assay should not exceed 0.5%.

3. Add 50 µl of diluted compound to “Stimulated” wells.

4. Add 50 µl of Diluent Solution to the “Unstimulated Control”.

5. Add 100 µl of Assay Medium to the “Cell-Free Control” wells.

6. Incubate cells at 37°C in a CO_2 incubator overnight.

7. Add 10 µl of 100 µM forskolin in Assay Medium to “Stimulated” wells (final concentration of forskolin is 10 µM).

8. Add 10 µl of Assay Medium to the “Unstimulated Control” and “Cell-Free Control” wells.

9. Incubate the plate at 37°C in a CO_2 incubator for 5-6 hours.

10. Add 110 µl of ONE-Step™ Luciferase reagent to each well.

11. Rock at RT for ~15 minutes.

12. Measure luminescence using a luminometer.

13. Data Analysis: Subtract average background luminescence (cell-free control wells) from luminescence reading of all wells. The fold induction of CRE luciferase reporter expression is the average background-subtracted luminescence of the stimulated wells divided by the average background-subtracted luminescence of the unstimulated control wells.

$$Fold\ induction = \frac{(luminescence\ of\ stimulated\ cells - average\ background)}{(average\ luminescence\ of\ unstimulated\ cells - average\ background)}$$

For research use only. Not for human, drug, diagnostic, or therapeutic use.
● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com
BPS Bioscience

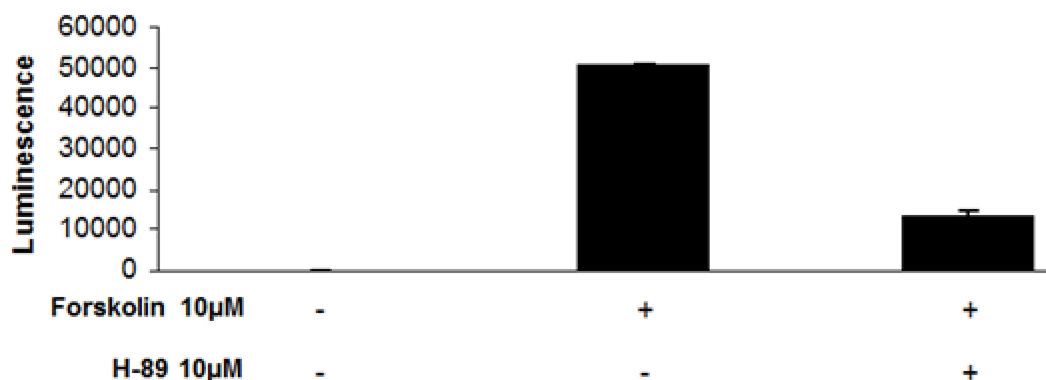

Figure 2. Inhibition of forskolin-induced reporter activity by H-89 in CRE/CREB Luciferase Reporter HEK293 Cell Line.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

References:

Montminy M.R., *et al.,* 1987 *Nature* 328(6126): 175-178.
Fan Chung K., 2006 *Eur. J. Pharmacol*. 533(1-3): 110-117.
Malik R., *et al.,* 2008 *Appl. Microbiol. Biotechnol.* 77 (5): 1167-1173.

License Disclosure

Visit bpsbioscience.com/license for the label license and other key information about this product.

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*
CRE/CREB Reporter Kit (cAMP/PKA Signaling Pathway)	60611	500 reactions
CRE/CREB Luciferase Reporter Jurkat Cell Line (cAMP/PKA Signaling Pathway)	79636	2 vials
CRE/CREB Luciferase Reporter CHO Cell Line (cAMP/PKA Signaling Pathway)	78568	2 vials
GLP-1R/CRE Luciferase Reporter HEK293 Cell Line	78176	2 vials
CGPRP/CRE Luciferase Reporter HEK293 Cell Line	78325	2 vials
GIPR/CRE Luciferase Reporter HEK293 Cell Line	78589	2 vials

Version 112223

BPS Bioscience
For research use only. Not for human, drug, diagnostic, or therapeutic use.
● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com