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Data Sheet

cAMP/PKA Signaling Pathway
CRE/CREB Reporter (Luc) – HEK293 Cell Line
Catalog #: 60515

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.

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.

Application
- Monitor cAMP/PKA signaling pathway activity.
- Screen activators or inhibitors of cAMP/PKA signaling pathway.

Format
Each vial contains ~1.5 X 10^6 cells in 1 ml of 10% DMSO.

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.

Storage
Immediately upon receipt, store in liquid nitrogen.

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General Culture conditions

Cells should be grown at 37°C with 7% CO_2 using MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Invitrogen #26140-079), 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone SV30010.01), and 100 µg/ml of Hygromycin B (Hyclone #SV30070.01). If culturing cells in medium from other vendors, it may be required to lower the percentage of CO_2 in the incubator depending on the $NaHCO_3$ level in the basal medium.

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 HEK293 Cell Thaw Medium (BPS Cat #60187), spin down cells (1000 rpm), and resuspend cells in pre-warmed HEK293 Cell Thaw Medium (BPS Cat #60187). Transfer resuspended cells to a single T25 flask and culture at 37°C in a 7% CO_2 incubator. At first passage, switch to HEK293 Cell Thaw Medium (BPS Cat #60187) containing Hygromycin. Cells should be split before they reach complete confluence.

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

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with Trypsin/EDTA. Add complete growth medium and transfer to a tube, spin down cells, and resuspend in freezing medium (10% DMSO + 90% FBS). Place at -80°C overnight and place in liquid nitrogen the next day. Alternatively, vials may be placed directly in liquid nitrogen.

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.

Functional Validation and Assay Performance

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.

Materials Required but Not Supplied

- HEK293 Cell Thaw Medium (BPS Cat #60187)
- Forskolin (LC Laboratories #F-9929), 10 mM in DMSO: activator of cAMP expression
- H-89 (Enzo Life Sciences # BML-EI196-0005) 10 mM in DMSO: inhibitor of cAMP/PKA pathway
- Assay medium: MEM medium (Hyclone #SH30024.01) + 10% FBS + 1% non-essential amino acids + 1 mM Na pyruvate + 1% Pen/Strep
- 96-well tissue culture treated white clear-bottom assay plate (Corning #3610)
- ONE-Step™ Luciferase Assay System (BPS, Cat. #60690)
- Luminometer

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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 and seed cells at a density of ~30,000 cells per well in 45 µl of assay medium into a white clear-bottom 96-well microplate.

2. Incubate cells at 37°C in a CO_2 incubator overnight (~ 16-18 hours).

3. Add 5 µl of threefold serial dilution of Forskolin in assay medium to stimulated wells.
   Add 5 µl of assay medium to the unstimulated control wells.
   Add 50 µl of assay medium to cell-free control wells (for determining background luminescence).
   Set up each treatment in at least triplicate.

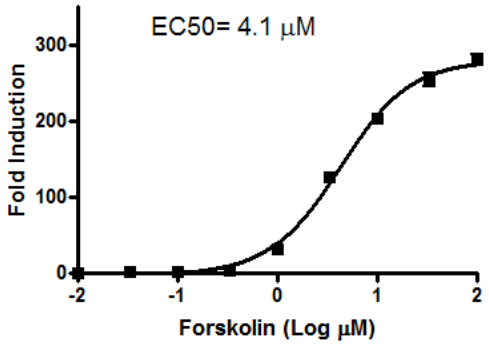
4. Incubate the plate at 37°C in a CO_2 incubator for 5-6 hours.

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 ~10 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.

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.



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B. Inhibition of Forskolin-induced reporter activity by an inhibitor of cAMP/ PKA signaling pathway in *cAMP/PKA Signaling Pathway CRE/CREB reporter (Luc)-HEK293 cells*

1. Harvest CRE/CREB reporter (Luc)-HEK293 cells from culture in Growth medium and seed cells at a density of ~30,000 cells per well in 40 µl of assay medium into a white clear-bottom 96-well microplate.

2. Add 5 µl of inhibitor in assay medium to inhibited wells.
   Add 5 µl of assay medium to the uninhibited control wells.
   Set up each treatment in at least triplicate.

   Incubate cells at 37°C in a CO_2 incubator overnight (~ 16-18 hours).

3. Add 5 µl of diluted Forskolin in assay medium to stimulated wells (final Forskolin concentration = 10 µM).
   Add 5 µl of assay medium to the unstimulated control wells.
   Add 50 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 ~10 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.

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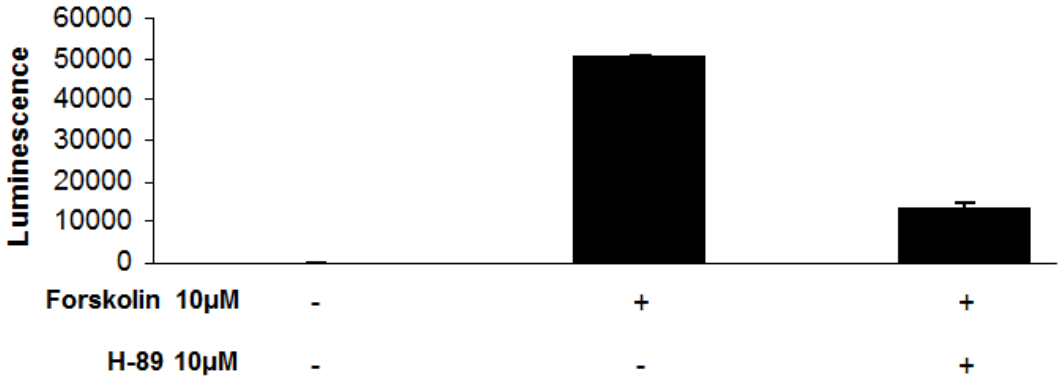
Figure 2. Inhibition of Forskolin-induced reporter activity by H-89 in CRE/CREB reporter (Luc)-HEK293 cells



Reference(s)

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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