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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<sup>6</sup> cells in 1 ml of 10% DMSO.

#### **Culture conditions**

Cells should be grown at 37°C with 7% CO<sub>2</sub> 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<sub>2</sub> in the incubator depending on the NaHCO<sub>3</sub> level in the basal medium.

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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 growth medium *without Hygromycin*, spin down cells (1000 rpm), and resuspend cells in pre-warmed growth medium *without Hygromycin*. Transfer resuspended cells to a single T25 flask and culture at 37°C in a 7% CO<sub>2</sub> incubator. At first passage, switch to growth medium 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.

*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**

- 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-Glo™ luciferase assay system (Promega #E6110) or other luciferase reagents for measuring firefly luciferase activity
- Luminometer

#### **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<sub>2</sub> incubator overnight (~ 16-18 hours).

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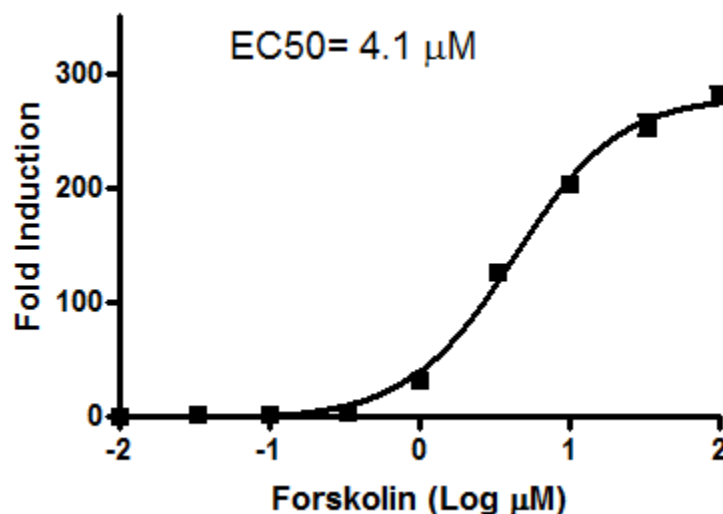
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3. Add 5  $\mu$ l of threefold serial dilution of Forskolin in assay medium to stimulated wells.  
 Add 5  $\mu$ l of assay medium to the unstimulated control wells.  
 Add 50  $\mu$ l of assay medium to cell-free control wells (for determining background luminescence).  
 Set up each treatment in at least triplicate.
4. Incubate the plate at 37°C in a CO<sub>2</sub> incubator for 5-6 hours.
5. Perform luciferase assay using ONE-Glo™ luciferase assay system: Add 50  $\mu$ l of ONE-Glo™ 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.

The EC<sub>50</sub> of Forskolin is ~ 4.1  $\mu$ M.



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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  $\mu$ l of assay medium into a white clear-bottom 96-well microplate.
2. Add 5  $\mu$ l of inhibitor in assay medium to inhibited wells.  
Add 5  $\mu$ l of assay medium to the uninhibited control wells.  
Set up each treatment in at least triplicate.

Incubate cells at 37°C in a CO<sub>2</sub> incubator overnight (~ 16-18 hours).

3. Add 5  $\mu$ l of diluted Forskolin in assay medium to stimulated wells (final Forskolin concentration = 10  $\mu$ M).  
Add 5  $\mu$ l of assay medium to the unstimulated control wells.  
Add 50  $\mu$ l 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<sub>2</sub> incubator for 5-6 hours.
5. Perform luciferase assay using ONE-Glo™ luciferase assay system: Add 50  $\mu$ l of One-Glo™ 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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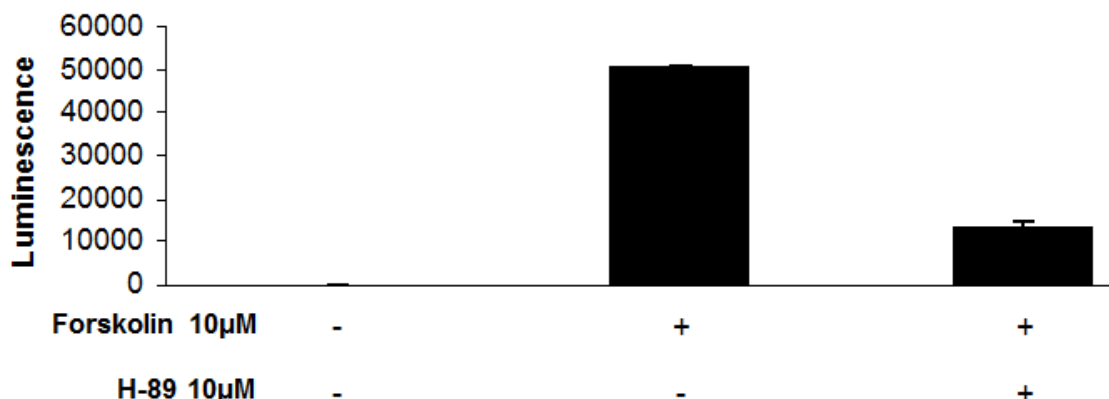
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**Figure 2. Inhibition of Forskolin-induced reporter activity by H-89 in CRE/ CREB reporter (Luc)-HEK293 cells**



#### **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.

#### **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.

#### **License Disclosure**

Purchase of this cell line grants you with a 10-year license to use this cell line in your immediate laboratory, for research use only. This license does not permit you to share, distribute, sell, sublicense, or otherwise make the cell line available for use to any other person, group, laboratory, department, research institution, hospital, university, or biotech company. The license does not permit the use of this cell line in humans or for therapeutic or drug use. Inappropriate use or distribution of this cell line will result in revocation of the license and result in an immediate cease of sales and distribution of BPS products to your laboratory. Publications using this cell line should reference BPS Bioscience, Inc., San Diego.

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