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Data Sheet

CRE/CREB Reporter (Luc) - Jurkat Cell Line

(cAMP/PKA Signaling Pathway)

Catalog #: 79636

Background

The CRE/CREB Reporter (Luc) – Jurkat 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 CRE/CREB Reporter (Luc) – Jurkat Cell Line contains a firefly luciferase gene under the control of multimerized cAMP response element (CRE) stably integrated into Jurkat 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.

Application

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

Format

Each vial contains ~2 x 10^6 cells in 1 ml of 10% DMSO

Storage

Immediately upon receipt, store in liquid nitrogen.

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.

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General Culture Conditions
Thaw Medium 2 (BPS Bioscience #60184): RPMI1640 medium (Life Technologies, #A10491-01) supplemented with 10% FBS (Life Technologies, #26140-079), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01).

Growth Medium 2E (BPS Bioscience #79638): Thaw Medium 2 (BPS Bioscience, #60184) plus 0.5 µg/ml of Puromycin (Takara, #631306).

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 2E.

It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 2, **(no Puromycin)**. Spin down the cells, remove supernatant and resuspend cells in pre-warmed Thaw Medium 2, **(no Puromycin)**. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator. After 24 hours of culture, add an additional 3 – 4 ml of Thaw Medium 2, **(no Puromycin)**. At first passage, switch to Growth Medium 2E **(contains Puromycin)**. Cells should be split before they reach 2.5 x 10^6 cells/ml.

To passage the cells, dilute cell suspension into new culture vessels at no less than 0.1 x 10^6 cells/ml. Subcultivation ratio: 1:5 to 1:10 twice a week.

Assay Performance
The following assays are designed for 96-well format. To perform the assay in different tissue culture formats, cell number and reagent volume should be scaled appropriately. All assay conditions should be tested in at least triplicate.

Materials Required but Not Supplied
- Forskolin (Sigma, #F3917)
- Assay Medium: Thaw Medium 2 (BPS Bioscience, #60184)
- Growth Medium 2E (BPS Bioscience, #79638)
- 96-well tissue culture treated white clear-bottom assay plate (Corning, #3610)
- One-Step luciferase assay system (BPS Bioscience, #60690)
- Luminometer

Forskolin dose response
1. Harvest CRE/CREB reporter (Luc)-Jurkat cells from culture in Growth Medium 2E and seed cells at a density of ~40,000 cells per well into white opaque 96-well microplate in 90 µl of assay medium.

2. Prepare threefold serial dilution of Forskolin in assay medium. Add 10 µl of diluted Forskolin to the stimulated wells.

3. Add 10 µl of assay medium to the unstimulated control wells (for measuring uninduced level of CRE/CREB reporter activity).

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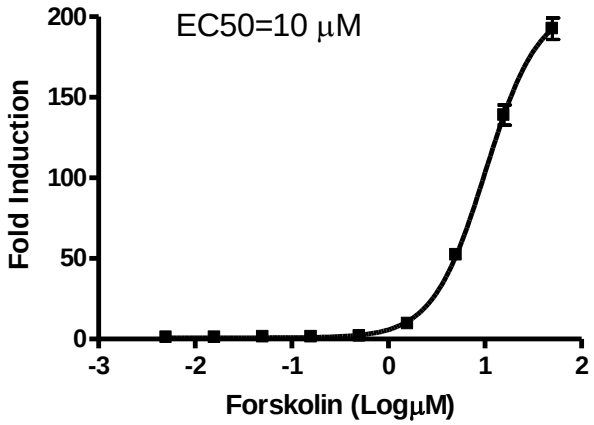
4. Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).

5. Incubate at 37°C with 5% CO_2 for 5-6 hours.

6. Prepare ONE-Step™ Luciferase Assay reagent according to recommended instructions. Add 100 µl of ONE-Step™ Luciferase Assay reagent per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer.

Figure 1. Forskolin dose response in CRE/CREB reporter (Luc)-Jurkat cells. Cells were treated with Forskolin for ~ 6 hours. The results were shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing values against the mean value for control cells without Forskolin treatment.



Related Products

Product	Cat. #	Size
CRE/CREB reporter (Luc) - HEK293 Cell line	60515	2 vials
PDE7A/CRE Reporter - HEK293 Recombinant Cell Line	60413	2 vials
CRE/CREB Reporter Kit (cAMP/PKA Signaling Pathway)	60611	500 rxns
CRE/CREB Transient Pack (cAMP/PKA Cell Signaling Pathway)	79267	500 rxns
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
Thaw Medium 2	60184	100 ml
Growth Medium 2E	79638	500 ml

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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.

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