6405 Mira Mesa Blvd Ste 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

Data Sheet

CRE/CREB Luciferase Reporter Lentivirus

Catalog #: 79580

Product Description

The main role of the cAMP response element, or CRE, is mediating the effects of Protein Kinase A (PKA) by way of transcription. Upon phosphorylation, CREB forms a functionally active dimer that binds the CRE element within the promoters of target genes and activates transcription. CRE is at the focus 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.

The CRE/CREB Luciferase Reporter Lentivirus are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to be transduced into almost all types of mammalian cells, including primary and non-dividing cells. The particles contain a firefly luciferase gene driven by multimerized cAMP response element (CRE) located upstream of the minimal TATA promoter (Figure 1). After transduction, activation of the cAMP/PKA signaling pathway in the target cells can be monitored by measuring the luciferase activity.

Application

- Screen for activators or inhibitors of cAMP/PKA signaling pathway in the transduced target cells
- Generation of CRE/CREB Luciferase Reporter stable cell line

Formulation

The lentiviruses were produced from HEK293T cells in medium containing 90% DMEM + 10% FBS.

Titer

Two vials (500 µl x 2) of CRE/CREB luciferase reporter lentivirus at a titer 1 x 10^7 TU/ml. The titer will vary with each lot; the exact value is provided with each shipment.

Storage

Lentiviruses are shipped with dry ice. For long term storage, it is recommended to store the virus at -80°C. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

Biosafety

The lentiviruses are produced with the second generation SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and integration into the genomic DNA of the target cells. None of the HIV genes (gag, pol, rev) will be expressed in the transduced cells, as they are expressed from packaging plasmids lacking the packing signal.

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Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.

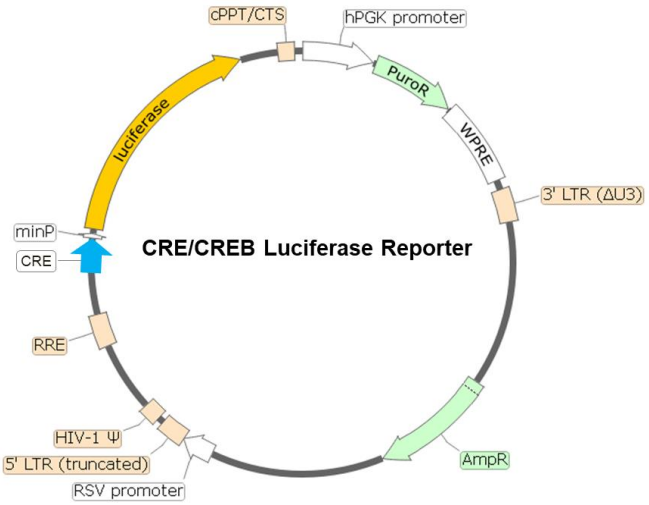

Figure 1. Schematic of the lenti-vector used to generate the CRE/CREB luciferase reporter lentivirus

Materials Required but Not Supplied

- Forskolin (Sigma, #F3917)
- HEK293 Growth Medium or use
  Thaw Medium 9 (BPS Bioscience #79665): MEM with 10% FBS, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate
- Polybrene (Millipore, #TR-1003-G)
- 96-well tissue culture treated white clear-bottom assay plate (Corning, #3610)
- One-Step luciferase assay system (BPS Bioscience, #60690)
- Luminometer

Assay Protocol

The following protocol is a general guideline for transducing HEK293 cells using CRE/CREB luciferase reporter lentivirus. The optimal transduction conditions (e.g. MOI, concentration of polybrene, time of assay development) should be optimized according to the cell type and the assay requirements. In most cell types, the expression of the reporter gene can be measured approximately 72 hours after transduction. For cell types with low transduction efficacy, it may be necessary to select the cells stably expressing the reporter gene with puromycin prior to carrying out the reporter assays.

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1. Day 1: Harvest HEK293 cells from culture and seed cells at a density of 5,000-10,000 cells per well into white opaque 96-well microplate in 50 µl of Thaw Medium 9 (BPS Bioscience #79665). Incubate cells at 37°C with 5% CO_2 overnight.

2. Day 2: To each well add 10 µl of CRE/CREB luciferase reporter lentivirus. Add polybrene to each well at a final concentration of 5 µg/ml. Gently swirl the plate to mix. Incubate the plate at 37°C with 5% CO_2 for 18-24 hours.
   Alternatively, seeding cells and the transduction can be performed at the same day.

3. Day 3: Remove the medium containing the lentivirus from the wells. Add 100 µl of fresh Thaw Medium 9 (BPS Bioscience #79665) to each well.

   If neither the polybrene nor the lentivirus adversely affects the target cells, it is not necessary to change the medium on Day 3. The target cells can be incubated with the virus for 48-72 hours before changing medium.

4. On the morning of Day 5, prepare diluted Forskolin in Thaw Medium 9 (BPS Bioscience #79665). Add 10 µl of diluted Forskolin to the Forskolin-stimulated wells. Add 10 µl of Thaw Medium 9 (BPS Bioscience #79665) to the unstimulated control wells (for measuring the uninduced level of CRE/CREB reporter activity).

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

6. Prepare the ONE-Step™ Luciferase reagent per recommended protocol. 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.

Important Notes:

1. To generate the CRE/CREB luciferase reporter stable cell line, on day 4 remove Thaw Medium 9 (BPS Bioscience #79665) and replace it with fresh growth medium containing the appropriate amount of puromycin for antibiotic selection of transduced cells.

2. The following Lenti Reporter Controls are also available from BPS Bioscience to meet your experimental needs:

   1) Reporter Negative Control Lentivirus (BPS Bioscience, #79578): Ready-to-transduce lentiviral particles expressing firefly luciferase under the control of a minimal promoter. The negative control is important to establish the specificity of any treatments and to determine the background reporter activity.

   2) Renilla Luciferase (Rluc) Lentivirus (BPS Bioscience, #79565): Ready-to-transduce lentiviral particles expressing Renilla luciferase under the CMV promoter. The RLuc lentivirus can serve as an internal control to overcome sample-to-sample variability when performing dual-luciferase reporter assays.

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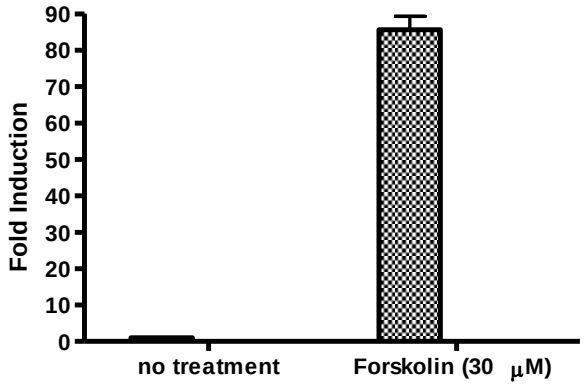

Figure 2. CRE/CREB luciferase reporter activity stimulated by Forskolin in HEK293 cells. Appropriate 10,000 HEK293 cells/well were transduced with 100,000 TU/well CRE/CREB luciferase reporter lentivirus. After 48 hours of transduction, medium was changed to Thaw Medium 9 (BPS Bioscience #79665). After 66 hours of transduction, cells were treated with 30 μM Forskolin for ~6 hours. The results are 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.

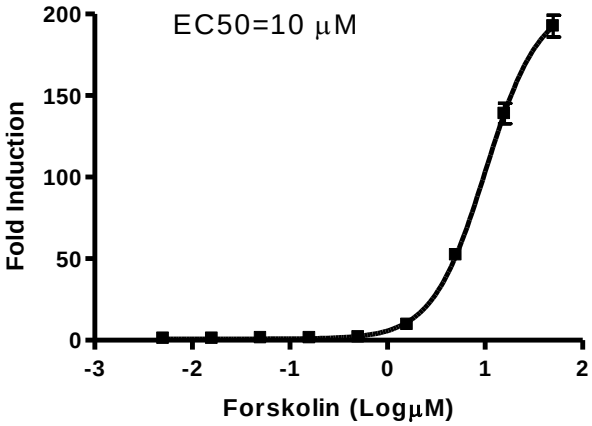

Figure 3. Generation of a stable CRE/CREB luciferase reporter Jurkat cell line using CRE/CREB luciferase reporter lentivirus. The CRE/CREB luciferase reporter Jurkat stable cell line was generated by transduction of Jurkat cells with CRE/CREB luciferase reporter lentivirus, followed by selection of a clonal cell line that has integrated the CRE/CREB luciferase reporter into the chromosome. Stimulation of the CRE/CREB reporter luciferase Jurkat stable cell line using Forskolin results in ~200-fold induction in CRE/CREB reporter activity.

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Related Products

Product	Cat. #	Size
NF-κB Luciferase Reporter Lentivirus	79564	500 µl x2
NFAT Luciferase Reporter Lentivirus	79579	500 µl x2
STAT3 Luciferase Reporter Lentivirus	79744	500 µl x2
STAT5 Luciferase Reporter Lentivirus	79745	500 µl x2
TCF/LEF Luciferase Reporter Lentivirus	79787	500 µl x2
Negative Control Lentivirus	79578	500 µl x2
Renilla Luciferase (Rluc) Lentivirus	79565	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (G418)	79692-G	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (Hygromycin)	79692-H	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (Puromycin)	79692-P	500 µl x2
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
Dual Luciferase (Firefly-Renilla) Assay System	60683	10 ml

References

1. Pessara, U., Koch, N. (1990) Tumor necrosis factor alpha regulates expression of the major histocompatibility complex class II-associated invariant chain by binding of an NF-κB-like factor to a promoter element. *Mol Cell Biol*. **10(8):**4146-4154.
2. Baeuerle, P.A. (1998) Pro-inflammatory signaling: last pieces in the NF-κB puzzle? *Curr Biol*. **8(1):**R19-R22.