ScienCell™ Research Laboratories

CRE/CREB GFP Reporter Vector

(cAMP/PKA/CREB Signaling Pathway)

(CR-GFP)

Catalog #VT009

100 transfections 96-well

The CRE/CREB (cAMP Response Element/cAMP Response Element-Binding Protein) signaling pathway regulates the transcription of genes involved in cell proliferation, survival, metabolism, neuronal plasticity, inflammation, and hormone responses. Upon activation by extracellular stimuli that elevate intracellular cAMP levels, protein kinase A (PKA) phosphorylates CREB at Ser133. Phosphorylated CREB binds to cAMP response elements (CREs) within target gene promoters and recruits transcriptional coactivators such as CBP/p300, resulting in transcriptional activation. The CRE/CREB GFP Reporter Vector is a plasmid-based fluorescent reporter for real-time monitoring of CREB-dependent transcription in mammalian cells. It contains multimerized cAMP response elements (CREs) upstream of GFP (Green Fluorescent Protein), allowing pathway activation to induce GFP expression for direct visualization of CREB signaling in living cells without cell lysis or additional detection reagents [1–3].

Kit Components

Cat #	Component	Quantity	Storage
VT009	CRE/CREB GFP Reporter Vector, lyophilized	1 vial	-20°C
GQ100-0.5	Nuclease-free H_2O	1vial (0.5mL)	4°C

Note: These vectors are ready for transient transfection. They are NOT MEANT for transformation and amplification in bacteria.

Additional Materials Required (Materials Not Included in Kit)

Product Name
Mammalian cell line of interest
Appropriate cell culture media
Transfection reagents suitable for your cell type (e.g., UniFectagen, Cat#0983)
Cell culture plates (e.g., 6-well, 12-well, 24-well or 96-well plates)
Serum-free or reduced for transfection (e.g., Transfection Max Medium, TMM - Cat#9051)
Forskolin
Fluorescence microscope or fluorescence plate reader

Quality Control

Each batch of CRE/CREB GFP Reporter Vector (CR-GFP) is verified for plasmid DNA purity and sequence integrity by spectrophotometry and Sanger sequencing. Functional validation is performed by transient transfection in HEK293T cells followed by CREB pathway activation

using Forskolin (5 µM, 24 h). Inducible GFP expression is confirmed by fluorescence microscopy (Figure 1A).

Product Use
CR-GFP is for research use only and is not approved for human or animal use, or application in clinical or *in vitro* diagnostic procedures.

Shipping and Storage
The product is shipped at room temperature. Upon receipt, store at -20°C.

References
[1] Shaywitz, A. J., & Greenberg, M. E. (1999). CREB: A stimulus-induced transcription factor activated by a diverse array of extracellular signals. *Annual Review of Biochemistry*, 68, 821–861.
[2] Johannessen, M., Delghandi, M. P., & Moens, U. (2004). What turns CREB on? *Cellular Signalling*, 16, 1211–1227.
[3] Mayr, B., & Montminy, M. (2001). Transcriptional regulation by the phosphorylation-dependent factor CREB. *Nature Reviews Molecular Cell Biology*, 2, 599–609.

Procedure

Important Notes:
- *Protocol is optimized for HEK293T cells in a 6-well plate format using UniFectagen transfection reagent (Cat# 0983).*
- *Different reagent: optimize DNA: reagent ratio, incubation time, and complex formation per manufacturer instructions.*
- *Different plate formats: scale volumes and cell numbers by surface area ratios.*
- *Set up ≥3 technical triplicate per condition; prepare transfection cocktails for multiple wells to minimize pipetting errors.*

1. **Cell Seeding**
   Seed approximately 500,000 cells/well in 2 mL complete growth medium in a 6-well plate. Incubate overnight at 37°C in a 5% CO_2.

2. **Plasmid Reconstitution**
   2.1. Add 200 µl nuclease-free H_2O (Cat#GQ100-0.5) to **CRE/CREB GFP Reporter Vector** (lyophilized, Cat#VT009) to make a work solution. Aliquot and store at -20°C (manual defrost freezer). Avoid repeated freeze-and-thaw cycles.

3. **Transfection Mix (per well):**
   3.1. Prepare one well by mixing 40 µL of the reporter plasmid with 36 µL of nuclease-free H_2O or serum free medium (TMM, Cat#9051).
   3.2. Add 18 µL UniFectagen Reagent B (Cat#0983). Vortex gently and spin down.
   4.1. Add 48 µL of UniFectagen Reagent A (Cat#0983) to bring the total transfection mixture volume to 142 µL. Vortex for 5 seconds and spin down.
   4.2. Incubate the mixtures at room temperature for 20-30 minutes.

5. **Transfection**
   5.1. Add 142 µL of transfection mixture to each well containing 2 mL fresh culture

medium**.**

5.2. Gently mix into the medium, avoid to disturber cells seeding.

5.3. Incubate the cells for 24 hours at 37°C, 5% CO_2 before downstream analysis.
Note: Depending on the cytotoxicity of the transfection reagent and the sensitivity of the cells, a medium change 4–6 hours post-transfection may be performed.

6. Treatment

6.1. Apply treatments according to experimental groups:

- **Group 1:** Fresh medium only (Untreated)
- **Group 2:** Fresh medium + Forskolin (5 µM).

6.2. Incubate for 24 hours at 37°C.
Notes: *Forskolin directly activates adenylyl cyclase, increasing intracellular cAMP levels and activating PKA, which phosphorylates CREB at Ser133 [1-2].*
Phosphorylated CREB binds CRE elements and induces GFP expression from the reporter construct [1-2].
Forskolin is widely used as a robust pharmacological activator of the CRE/CREB signaling pathway [2].

7. Fluorescence Detection

7.1. Monitor GFP expression using fluorescence microscopy or a fluorescence plate reader. GFP expression reflects CRE/CREB pathway activation.

Data Analysis

Increased GFP fluorescence relative to untreated cells indicates activation of CRE/CREB signaling.

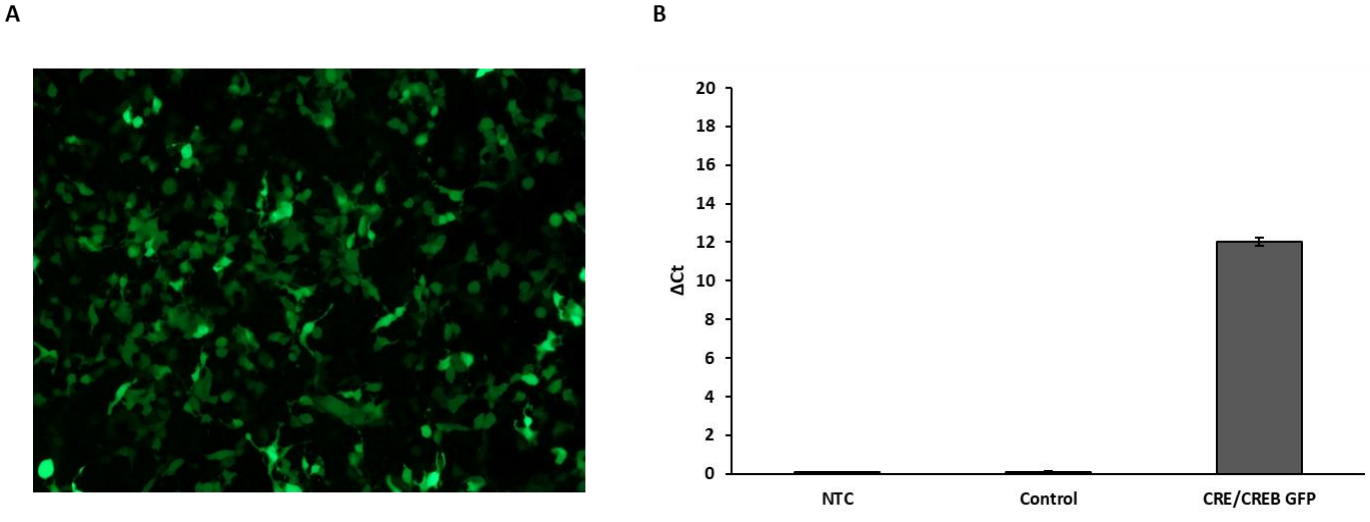

Figure 1. A. Representative fluorescence images of HEK293T cells transfected with the CRE/CREB GFP Reporter Vector showing CREB-dependent GFP expression following pathway activation. **B.** GFP expression was detected by RT-qPCR in transfected HEK293T cells (Ct ~25), with no signal in non-transfected controls. Data were normalized to GAPDH (ΔCt).