ScienCell™ Research Laboratories

CRE/CREB Dual Reporter Vector

(cAMP Response Pathway)

(CRERV)

Catalog #VT004

100 transfections 96-well

Product Description

The cAMP/CREB signaling pathway (cyclic adenosine monophosphate/cAMP response element-binding protein) mediates diverse cellular responses to hormones and neurotransmitters through cAMP elevation (AMP cyclic), PKA activation (Protein Kinase A), CREB phosphorylation, and binding to cAMP Response Elements (CREs) to drive target gene expression, with dysregulation implicated in metabolic disorders, neurodegeneration, and cancer. Forskolin or cAMP analogs activate this pathway by stimulating adenylyl cyclase and downstream CREB-dependent transcription. [1-2]. The CRE/CREB Dual Reporter Vector (CRERV) is a DNA-based dual-luciferase system engineered to monitor this cAMP/CREB–dependent transcriptional activity in mammalian cells. It comprises a CRE/CREB Reporter Mix, in which Firefly luciferase is driven by multimerized cAMP Response Elements and co-formulated with a constitutive Renilla luciferase plasmid for internal normalization, and a Negative Control Reporter Mix lacking CRE binding sites to define non-inducible background and pathway specificity. Firefly luciferase provides a strong ATP-dependent signal proportional to CREB-driven transcription, while Renilla luciferase offers an ATP-independent internal control, enabling accurate quantification of cAMP pathway activity suitable for pathway studies, genetic perturbation experiments, and screening of small-molecule or biologic modulators.

Kit Components

Cat #	Component	Quantity	Storage
VT004	CRE/CREB Reporter Vector Mix (CRE/CREB Firefly Luciferase Reporter Vector + constitutively Renilla Luciferase Vector), lyophilized	1 vial	-20°C
VTNEG-001	Negative Control Vector Mix (Non-Inducible Firefly Luciferase Vector +constitutively Renilla Luciferase Vector), lyophilized	1 vial	-20°C
GQ100-0.5	Nuclease-free H_2O (0.5 mL)	1 vial	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)
Nuclease-free H_2O
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)
White or opaque 96-well plate for luminescence reading
Luciferase Assay Kit (LAK, Cat#9048)
Forskolin
8-Br-cAMP
Luminometer

Quality Control

Each batch of CRE/CREB Dual Reporter Vector (CRERV) undergoes rigorous quality control to optimal performance. Plasmid DNA purity is verified by spectrophotometric, key regulatory elements and luciferase genes are sequence-validated by Sanger sequencing, and functional activity is confirmed via transient transfection in HEK293T cells and ± cAMP/CREB pathway activation (Forskolin, 5 μM, or 8-Br-cAMP, 0.5 mM, 6h), followed by Luciferase Assay Kit (LAK, Cat#9048) to verify expected Firefly and Renilla luminescence signals (Figure 1).

Product Use

CRERV 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 AJ, Greenberg ME. CREB: A stimulus-induced transcription factor activated by a diverse array of extracellular signals. *Annual Review of Biochemistry*. 1999; 68:821–861.

[2] Sands WA, Palmer TM. Regulating gene transcription in response to cyclic AMP elevation. *Biochemical Society Transactions*. 2008;36(Pt 3): 422-426.

Procedure

Important Notes:

- *Protocol is optimized for HEK293T cells in a 96-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 30,000 cells/well in 180 μL complete growth medium in a 96-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 Reporter Vector Mix** (lyophilized, Cat#VT004) to make a work solution. Aliquot and store at -20°C (manual defrost freezer). Avoid repeated freeze-and-thaw cycles.
   - **2.2.** Add 200 µl nuclease-free H_2O (Cat#GQ100-0.5) to **Negative Control Vector Mix** (lyophilized, Cat#VTNEG-001) to make a work solution. Aliquot and store at -20°C (manual defrost freezer). Avoid repeated freeze-and-thaw cycles.

3. **Preparation of DNA Complexes and Cell Transfection (per well)**
   - **3.1. Prepare Plasmid**
     - **A. CRE/CREB Reporter (Cat#VT004)**
       Prepare one well by mixing 2 µL of the reporter plasmid with 5 µL of nuclease-free H_2O or serum free medium (TMM, Cat#9051).
     - **B. Negative Control (Cat#VTNEG-001)**
       Prepare a separate well by mixing 2 µL of de control plasmid with 5 µL of nuclease-free H_2O or serum free medium (TMM, Cat#9051).

4. **Prepare Transfection Reagent Mix (per well)**
   - **4.1.** Add 0.5 µL of UniFectagen reagent B (Cat#0983) to each plasmid prepared in a 1.5mL sterile microcentrifuge tube. Vortex gently and spin down.
   - **4.2.** Add 2 µL of UniFectagen reagent A (Cat#0983) to make the total transfection mixture volume to 9.5 µL, vortex for 5 seconds and spin down.
   - **4.3.** Incubate at room temperature for 20-30min.
     Note: The optimal amount of transfection reagent may vary depending on the reagent and cell type; optimization is recommended.

5. **Transfection**
   - **5.1.** Add the 9.5 µL of transfection mixture to each well containing 100 µL fresh culture medium**.**
   - **5.2.** Gently mix in 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) or 8-Br-cAMP (0.5 mM)
   - **6.2.** Incubate for 6 hours at 37°C.

7. **Luciferase Assay**
   - **7.1.** At the chosen time point post-transfection and/or treatment, measure luciferase activity using the Luciferase Assay Kit (LAK, Cat#9048).
   - **7.2.** Follow the instructions provided in the kit manual for cell lysis, reagent preparation, and luminescence detection. Be sure to:
     - Use a compatible luminometer.

- Normalize per well: Firefly RLU (Relative Luminescence Units) ÷ Renilla RLU (same well).
- Use Negative Control Vector to assess background/non-specific effects.
- Calculate fold induction as Treatment ÷ Untreated (each reporter separately).

Dose Response of Effect of CRE/CREB Reporter Vector to Forskolin and 8-Br-cAMP

Day 1 – Cell Seeding

A. Plate 30,000 HEK293T cells/well in a 96-well plate in 180 µL of complete growth medium.

B. Incubate overnight at 37°C, 5% CO_2.

Day 2 – Transfection

A. Prepare two sets of well: **CRE/CREB Reporter Vector Mix (Cat#VT004)** and **Negative Control Vector Mix (Cat#VTNEG-001)**

A. Transfect using optimized 96-well conditions with UniFectagen (Cat# 0983) as described in Procedure section.

B. Incubate the cells for 16-24 hours at 37°C.

Day 3 –Treatment

- **Group 1:** Fresh medium only (Untreated)
- **Group 2:** Fresh medium + Forskolin (0.1-10 µM) and
- **Group 3:** Fresh medium + 8-Br-cAMP (0.1-3 mM)
- Incubate for 6 hours at 37°C.

Day 4 – Luciferase Assay Kit

- Perform assay using Luciferase Assay Kit (Cat#9048) per kit instructions.
- Normalize Firefly RLU ÷ Renilla RLU (same well).
- Calculate fold induction: Treatment ÷ Untreated.

Notes

- *Forskolin/8-Br-cAMP: Increase cAMP → CREB phosphorylation → CRE-dependent transcription [2].*
- *Forskolin/8-Br-cAMP treatment: Standard positive controls for robust CRE reporter activation in cultured cells [2].*
- *Use transfection reagent-compatible medium for optimal efficiency and cell viability.*

Data Analysis

To obtain normalized luciferase activity, calculate per well: Firefly RLU ÷ Renilla RLU (same well). Fold induction = Treatment ÷ Untreated (for each reporter separately).

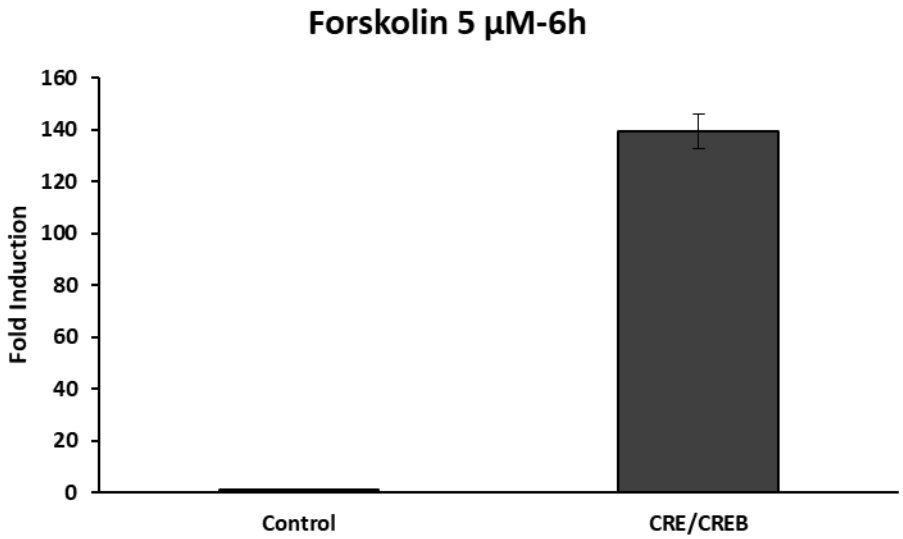

Figure 1. HEK293T cells transiently transfected CRE/CREB Dual Reporter Vector, treated 6h with Forskolin (5 µM). Firefly/Renilla normalized luciferase activity (mean ± SD, triplicates).

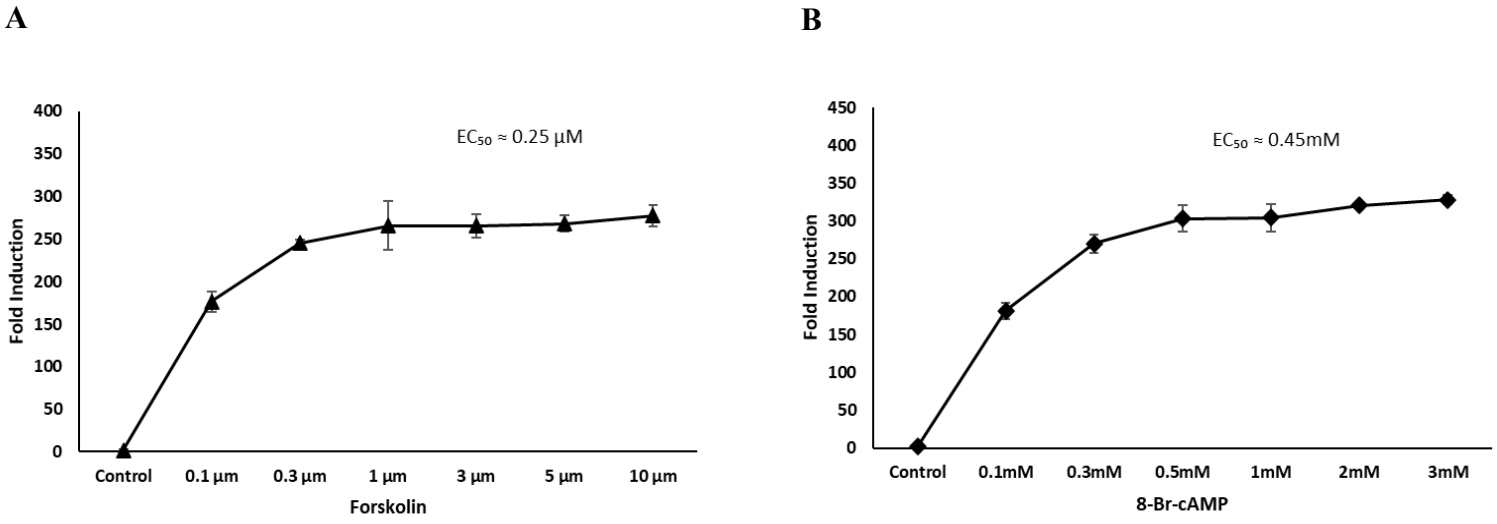

Figure 2. HEK293T cells transfected with CRE/CREB Dual Reporter Vector, treated 6h with indicated concentrations of Forskolin (**A**) and 8-Br-cAMP (**B**). Firefly/Renilla normalized luciferase activity shows dose-dependent CRE reporter induction by both agonists (mean ± SD, triplicates).