ScienCell™ Research Laboratories

AP-1 Dual Reporter Vector

(Activator Protein-1 Signaling Pathway)

(AP1RV)

Catalog#VT005

100 transfections 96-well

Product Information

The AP-1 signaling pathway (Activator Protein 1) integrates diverse extracellular signals through MAPK (Mitogen-Activated Protein Kinase) cascades (JNK -Jun N-terminal kinases, ERK - extracellular signal-regulated kinases, p38) leading to activation of Jun/Fos family transcription factors that bind AP-1 response elements (TRE) to regulate genes involved in proliferation, differentiation, apoptosis, and inflammation, with dysregulation implicated in cancer and fibrosis. PMA (Phorbol 12-myristate 13-acetate) or growth factors activate AP-1 through PKC/Ras/MAPK (Protein Kinase C/Rat Sarcoma virus/Mitogen-Activated Protein Kinase) pathways and downstream c-Fos/c-Jun dimerization [1-2]. The AP-1 Dual Reporter Vector (AP1RV) is a DNA-based dual-luciferase system engineered to monitor this AP-1–dependent transcriptional activity in mammalian cells. It comprises an AP-1 Reporter Mix, in which Firefly luciferase is driven by multimerized AP-1 response elements and co-formulated with a constitutive Renilla luciferase plasmid for internal normalization, and a Negative Control Reporter Mix lacking AP-1 binding sites to define non-inducible background and pathway specificity. Firefly luciferase provides a strong ATP-dependent signal proportional to AP-1–driven transcription, while Renilla luciferase offers an ATP-independent internal control, enabling accurate quantification of AP-1 pathway activity suitable for pathway studies, genetic perturbation experiments, and screening of small-molecule or biologic modulators.

Kit Components

Cat #	Component	Quantity	Storage
VT005	AP-1 Reporter Vector Mix (AP-1 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)	1vial	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)
Phorbol 12-myristate 13-acetate (PMA)
Luminometer

Quality Control

Each batch of AP-1 Dual Reporter Vector (AP1RV) 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 ± MAPK/AP-1 pathway activation (PMA 50 nM, 6h), followed by Luciferase Assay Kit (LAK, Cat#9048) to verify expected Firefly and Renilla luminescence signals (Figure 1).

Product Use

AP1RV 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] Bernard A, et al. Genome-scale functional profiling of the mammalian AP-1 signaling pathway. *Proc Natl Acad Sci U S A*. 2003;100(21):12153-12158.

[2] Sathya S, et al. Forskolin and phorbol 12-myristate 13-acetate modulate AP-1 factors and cell cycle regulators in MCF-7 cells. *J Cell Biochem*. 2019;120(4):5427-5439.

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 **AP-1 Reporter Vector Mix** (lyophilized, Cat#VT005) 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**
   - **3.1. Prepare Plasmid (per well)**
     - **A. AP-1 Reporter (Cat#VT005)**
       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 the 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 to UniFectagen reagent B (Cat#0983) into each plasmid prepared into a 1.5mL sterile microcentrifuge tube. Vortex gently and spin down.
   - **4.2.** Add 2 µL to UniFectagen reagent A (Cat#0983) to bring 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 for 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 + PMA (50 nM)
   - **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 AP-1 Dual Reporter Vector to PMA

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: **AP-1 Reporter Vector Mix (Cat#VT005)** and **Negative Control Vector Mix (Cat#VTNEG-001).**
- **B.** Transfect using optimized 96-well conditions with UniFectagen (Cat# 0983) as described in Procedure section.
- **C.** Incubate the cells for 16-24 hours at 37°C.

Day 3 –Treatment

- Group 1: Fresh medium only (Untreated)
- Group 2: Fresh medium + PMA (3-100 nM)
- Incubate for 6 hours at 37°C.

Day 4 – Luciferase Assay

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

Notes

- *PMA: Directly activates PKC → MAPK phosphorylation (ERK/JNK) → AP-1 transcriptional activation [1-2].*
- *PMA treatment: Standard method for robust, rapid AP-1–dependent gene expression 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).

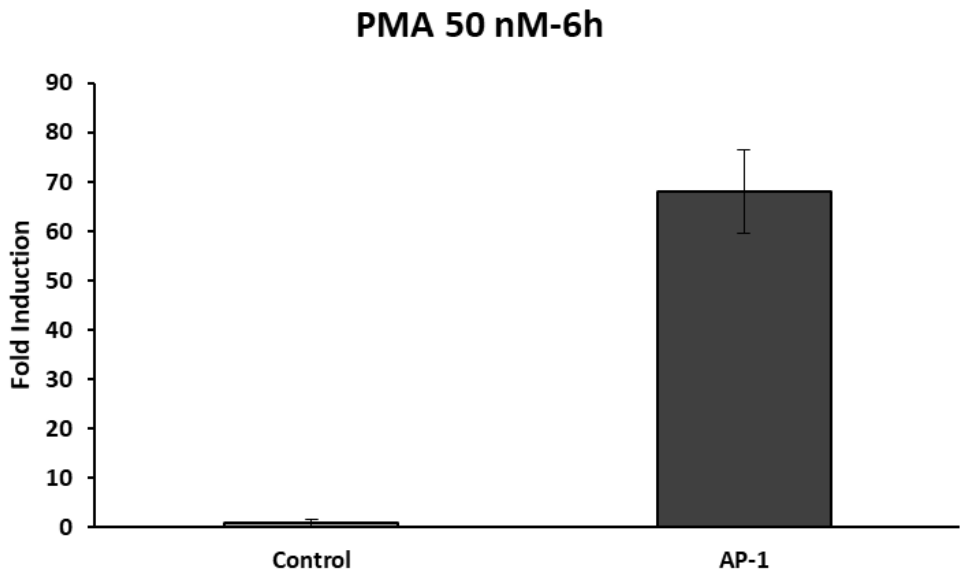

Figure 1. HEK293T cells transiently transfected with AP-1 Dual Reporter Vector, treated 6h with PMA (50 nM). Firefly/Renilla normalized luciferase activity (mean ± SD, triplicates).

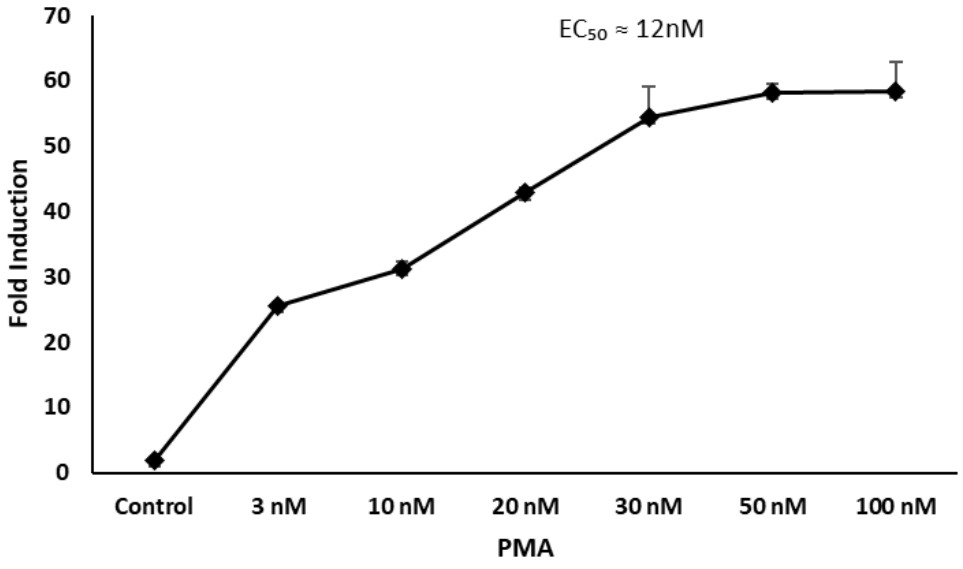

Figure 2. HEK293T cells transfected with AP-1 Dual Reporter Vector, treated 6h with indicated concentrations of PMA. Firefly/Renilla normalized luciferase activity shows dose-dependent AP-1 reporter induction by both agonists (mean ± SD, triplicates).