ScienCell™ Research Laboratories

# p53 RE Dual Reporter Vector

(p53 Signaling Pathway)

**(P53RV)**

Catalog #VT006

*100 transfections 96-well*

## Product Description

The p53 signaling pathway (*tumor protein p53*, a critical tumor suppressor transcription factor) acts as a critical tumor suppressor, responding to cellular stress (DNA damage, oncogene activation) through p53 stabilization, nuclear translocation, and binding to p53 response elements (REs) to transactivate genes involved in cell cycle arrest, DNA repair, and apoptosis, with p53 mutations being the most common genetic alteration in human cancers. Nutlin-3 or DNA-damaging agents activate this pathway by blocking MDM2-mediated p53 degradation or inducing post-translational modifications [1-3]. The p53 RE Dual Reporter Vector (P53RV) is a DNA-based dual-luciferase system engineered to monitor this p53-dependent transcriptional activity in mammalian cells. It comprises a p53 RE Reporter Mix, in which Firefly luciferase is driven by multimerized p53 response elements and co-formulated with a constitutive Renilla luciferase plasmid for internal normalization, and a Negative Control Reporter Mix lacking p53 RE binding sites to define non-inducible background and pathway specificity. Firefly luciferase provides a strong ATP-dependent signal proportional to p53-driven transcription, while Renilla luciferase offers an ATP-independent internal control, enabling accurate quantification of p53 pathway activity suitable for pathway studies, genetic perturbation experiments, and screening of small-molecule or biologic modulators.

## Kit Components

| Cat # | Component | Quantity | Storage |
|---|---|---|---|
| VT006 | p53 RE Reporter Vector Mix (p53 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) |
| Nutlin-3a |
| Cisplatin |
| Luminometer |

**Quality Control**

Each batch of p53 RE Dual Reporter Vector (P53RV) 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 ± p53 pathway activation (Nutlin-3a -0.3 μM, or Cisplatin 0.5 μM, overnight), followed by Luciferase Assay Kit (LAK, Cat#9048) to verify expected Firefly and Renilla luminescence signals (Figure 1).

**Product Use**

P53RV 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] El-Deiry WS, Kern SE, Pietenpol JA, Kinzler KW, Vogelstein B. (1992) "Definition of a consensus binding site for p53." *Nature Genetics*. 1(1):45–49.

[2] Vassilev L.T., et al. (2004) "In vivo activation of the p53 pathway by small-molecule antagonists of MDM2." *Science*. 303(5659):844–848.

[3] Menendez D, et al. (2009) "Redefining the p53 response element." *PNAS.* 106 (34) 14373-14378.

[4] Lalier L, Cartron PF, Olivier C, Logé C, Bougras G, Juin P, et al. (2011) "Activation of p53 by Nutlin-3a induces apoptosis and cellular senescence in glioma cells and synergizes with cisplatin." *PLOS ONE*. 6(4).

**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 **p53 RE Reporter Vector Mix** (lyophilized, Cat #VT006) 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. p53 RE Reporter (Cat#VT006)**
       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 to UniFectagen reagent B (Cat#0983) into each plasmid prepared into a 1.5mL sterile microcentrifuge tube. Vortex gently and spin down.
   - **4.2.** Then add 2 µL to 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 + Nutlin-3a (0.3 µM) or Cisplatin (0.5 µM)
   - **6.2.** Incubate overnight 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 p53 RE Reporter Vector to Nutlin-3a and cis-Platin**

**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 with 5% $CO_2$.

**Day 2 – Transfection**

**A.** Prepare two sets of well: **p53 RE Reporter Vector Mix (Cat#VT006)** 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 – Medium Change and Treatment**

- **Group 1:** Fresh medium only (Untreated)
- **Group 2:** Fresh medium + Nutlin-3a (0.1-10 µM)
- **Group 3:** Fresh medium + Cisplatin (0.05-1 µM)
- Incubate overnight 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*

- *Nutlin-3a: Inhibits MDM2-p53 interaction → prevents p53 degradation → stabilization, nuclear translocation, and p53 RE activation [1-4].*
- *Cisplatin treatment: Standard positive control for robust p53-dependent transcriptional activation in cultured cells [4].*
- *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).

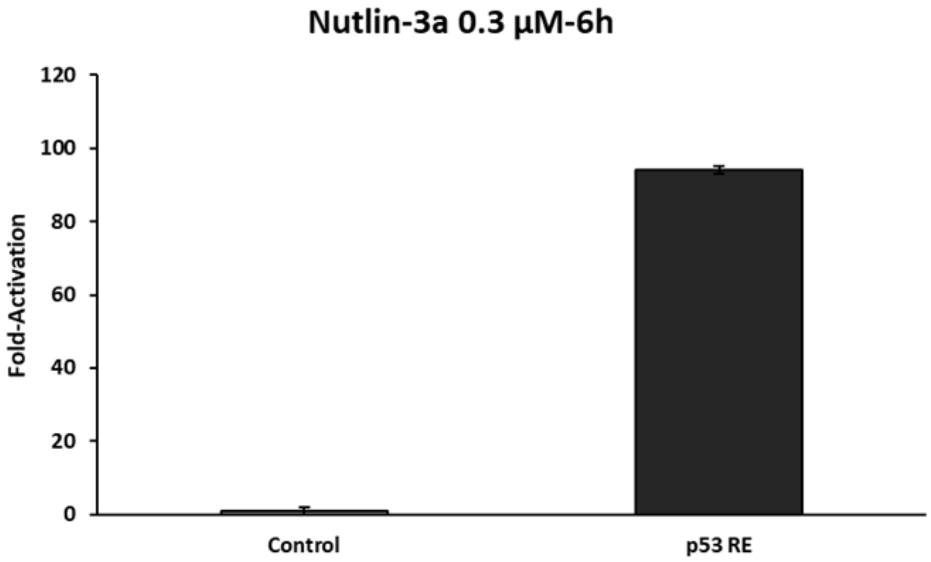


**Figure 1.** HEK293T cells transiently transfected with p53 RE Dual Reporter Vector, treated 6h with Nutlin-3a (0.3 µM). Firefly/Renilla normalized luciferase activity (mean ± SD, triplicates).

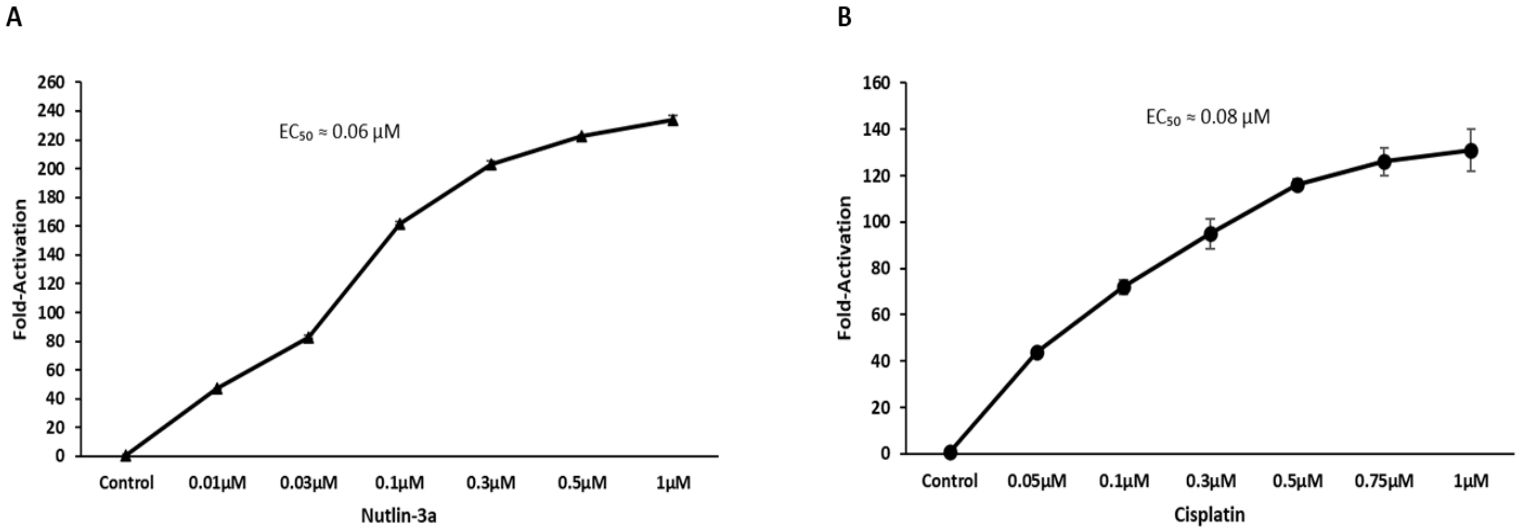


**Figure 2.** HEK293T cells transfected with p53 RE Dual Reporter Vector, treated 6h with indicated concentrations of Nutlin-3a (**A**) and Cisplatin (**B**). Firefly/Renilla normalized luciferase activity shows dose-dependent p53 reporter induction by both agonists (mean ± SD, triplicates).