HRE GFP Reporter Vector

(Hypoxia Response Pathway)

(HR-GFP)

Catalog #VT008

100 transfections 96-well

The HIF (Hypoxia-Inducible Factor) signaling pathway regulates cellular adaptation to low oxygen conditions and plays a central role in angiogenesis, metabolism, tumor progression, and tissue survival. Under hypoxic conditions, HIF-α subunits stabilize, accumulate in the cytoplasm, and translocate to the nucleus, where they dimerize with HIF-β and bind hypoxia response elements (HREs) to activate transcription of target genes. The HRE-GFP reporter vector is a plasmid-based fluorescent reporter for real-time monitoring of HIF-dependent transcription in mammalian cells; it contains multimerized hypoxia response elements upstream of GFP (Green Fluorescent Protein) so pathway activation induces GFP expression, enabling direct visualization of hypoxia signaling in living cells without lysis or additional detection reagents [1–3].

Kit Components

Cat #	Component	Quantity	Storage
VT008	HRE 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)
Cobalt Chloride ($CoCl_2$)
Fluorescence microscope or fluorescence plate reader

Quality Control

Each batch of HRE GFP Reporter Vector (HR-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 HIF-1 pathway activation using $CoCl_2$ (300 µM, 24h). Inducible GFP expression is confirmed by fluorescence microscopy. (Figure 1A).

Product Use
HR-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] Shibata, T., Giaccia, A. J., & Brown, J. M. (2000). Development of a hypoxia-responsive vector for tumor-specific gene therapy. *Gene Therapy*, 7(7), 493–498.
[2] Vordermark, D., Shibata, T., & Brown, J. M. (2001). Green fluorescent protein is a suitable reporter of tumor hypoxia despite an oxygen requirement for chromophore formation. *Neoplasia*, 3(6), 527–534.
[3] Wang, L., et al. (2024). Hypoxia-responsive HRE-GFP reporter system for monitoring tumor hypoxia and evaluating HIF-targeted gene therapy. *Journal of Cancer*, 15(14), 4345–4356.

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 **HRE GFP Reporter Vector** (lyophilized, Cat#VT008) 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 + $CoCl_2$ (200 µM)

6.2. Incubate for 24 hours at 37°C.
Notes: *$CoCl_2$: Inhibits PHDs → prevents HIF-1α degradation → nuclear accumulation and HRE activation [2-3].*
$CoCl_2$ treatment: Standard chemical hypoxia mimic for robust HIF transcriptional activation in cultured cells [2-3].

7. Fluorescence Detection

7.1. Monitor GFP expression using fluorescence microscopy or a fluorescence plate reader. GFP expression reflects HIF-1 pathway activation.

Data Analysis
Increased GFP fluorescence relative to untreated cells indicates activation of HIF-1 signaling.

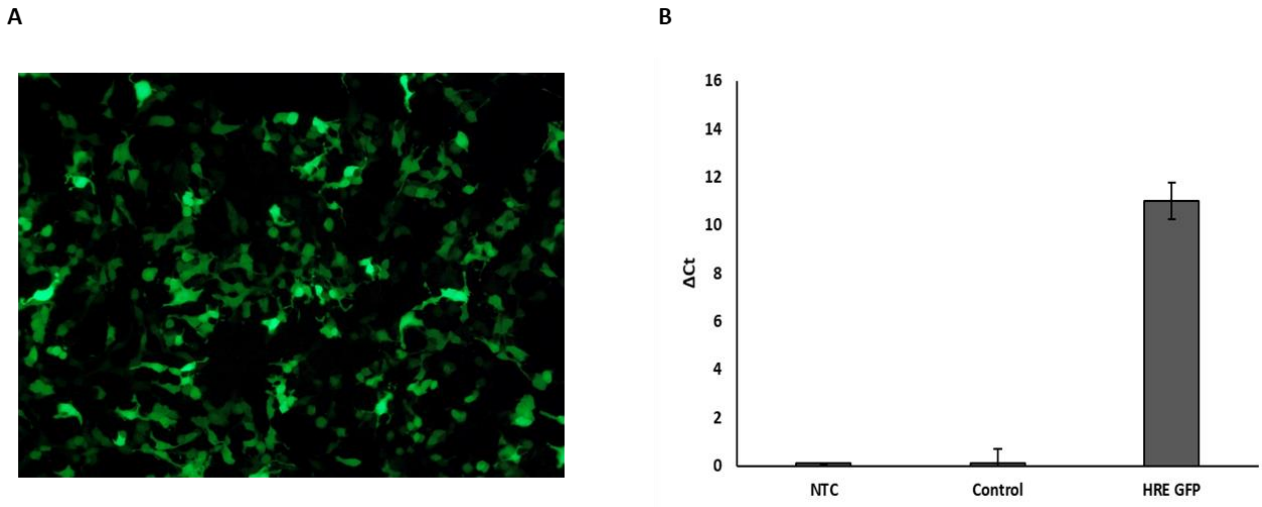

Figure 1. A. Representative fluorescence images of HEK293T cells transfected with the HRE GFP Reporter Vector showing HIF-1-dependent GFP expression following pathway activation. **B.** GFP expression was detected by RT-qPCR in transfected HEK293T cells (Ct ~27), with no signal in non-transfected controls. Data were normalized to GAPDH (ΔCt).