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Zuschläge

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- Trockeneiszuschlag
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Description

The Activator protein 1 (AP1) eGFP Reporter Lentiviruses are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to be transduced into almost all types of mammalian cells, including primary and non-dividing cells. The particles contain an eGFP gene driven by an AP1 response element located upstream of the minimal TATA promoter (Figure 1). After transduction, activation of the JNK signaling pathway and AP1-mediated activity in the target cells can be monitored by eGFP expression.

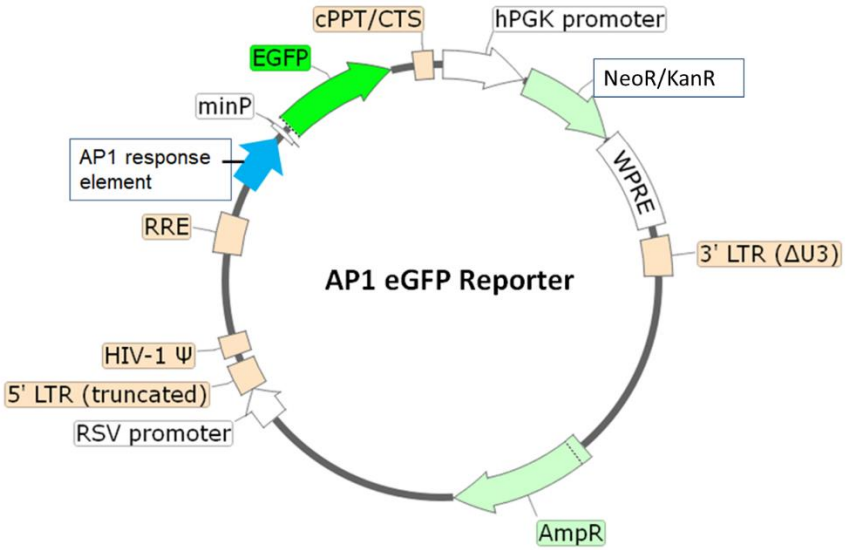

Figure 1. Schematic of the lenti-vector used to generate the AP1-eGFP Reporter Lentivirus.

Application(s)

- Screen for activators or inhibitors of the JNK signaling pathway in the transduced target cells.
- Generate an AP1 eGFP Reporter stable cell line (geneticin-resistant).

Background

The stress-activated protein kinase/c-jun N-terminal kinase (SAPK/JNK) family of proteins are activated by stress, inflammatory cytokines, mitogens, oncogenes, and inducers of cell differentiation and morphogenesis. Upon activation of the SAPK/JNK pathway, MAP Kinase Kinases phosphorylate and activate JNKs. The activated JNKs translocate to the nucleus where they phosphorylate and activate transcription factors such as c-Jun, c-Fos, ATF (Activating transcription factor), and JDP (Jun dimerization protein). These transcription factors form heterodimers termed AP1, which bind to AP1 response elements in the promoters of target genes and induce their transcription.

Formulation

The lentivirus particles were produced from HEK293T cells. They are supplied in cell culture medium containing 90% DMEM + 10% FBS.

Titer

Two vials (500 µl x 2) of lentivirus at a titer >10^7 TU/ml. The titer will vary with each lot; the exact value will be provided with each shipment.

Storage

Lentiviruses are shipped with dry ice. For long-term storage, it is recommended to store the lentiviruses at -80°C. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

Biosafety

The lentiviruses are produced with the third generation SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and integration into the genomic DNA of the target cells. None of the HIV genes (gag, pol, rev) will be expressed in the transduced cells, as they are expressed from packaging plasmids lacking the packing signal and are not present in the lentivirus particle. Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS Bioscience recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.

Materials Required but Not Supplied

These materials are not supplied with the lentivirus but are necessary to follow the protocol described in the "Validation Data" section. Media, reagents, and luciferase assay buffers used at BPS Bioscience are all validated and optimized for use with this lentivirus and are highly recommended for best results.

Name	Ordering Information
Phorbol 12-Myristate 13-Acetate (PMA)	LC Laboratories #P-1680
HEK293 growth medium or Thaw Medium 9	BPS Bioscience #79665
Assay Medium 1B	BPS Bioscience #79617
Polybrene	Millipore #TR-1003-G

Assay Protocol

The following protocol is a general guideline for transducing HEK293 cells using AP1 eGFP reporter lentivirus. The optimal transduction conditions (e.g., multiplicity of infection (MOI), concentration of polybrene, time of contact) should be optimized according to the cell type and the assay requirements. In most cell types, the expression of the reporter gene can be measured approximately 72 hours after transduction. For cell types with low transduction efficacy, it may be necessary to enrich the cells stably expressing the reporter gene with geneticin prior to carrying out the reporter assays.

1. **Day 1**: Seed HEK293 cells at a density of 5,000-10,000 cells per well into white, clear bottom 96-well microplate in 90 µl of Thaw Medium 9 (BPS Bioscience #79665).
    a. To each well, add 2 µl of AP1 eGFP reporter lentivirus. Optional: Add polybrene to each well at a final concentration of 5 µg/ml. Gently swirl the plate to mix. Incubate the plate at 37°C with 5% CO_2 for 48 hours.

2. **Day 3**: Remove the medium containing the lentivirus from the wells.
    a. Add 100 µl of Assay Medium 1B (BPS Bioscience #79617) containing PMA to stimulated wells.
    b. Add 100 µl of Assay Medium 1B to control untreated wells.

3. Incubate the plate at 37°C with 5% CO_2 for 18-24 hours, observe eGFP expression under fluorescence microscopy.

Validation Data

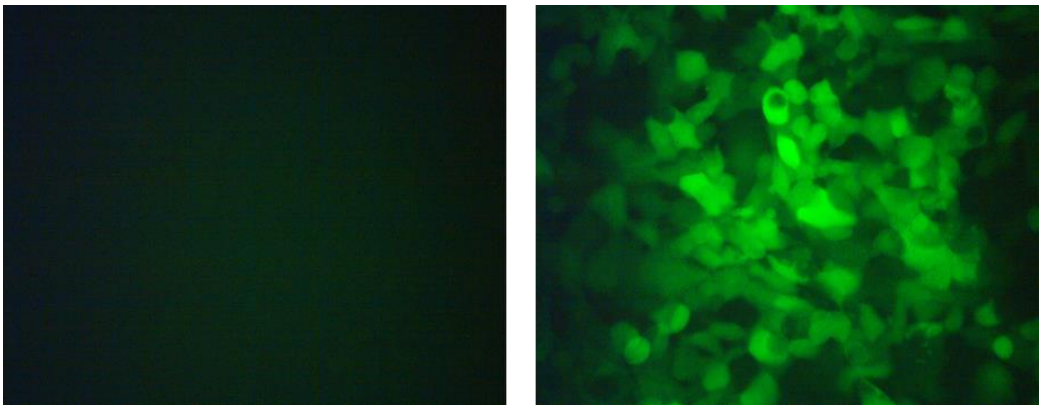

Figure 2. AP1 eGFP reporter expression stimulated by PMA in HEK293 cells.
Approximately 10,000 HEK293 cells/well were transduced with 100,000 TU/well AP1 eGFP reporter lentivirus. After 66 hours of transduction, the medium was changed to assay medium 1B, and the cells were treated with 50 nM of PMA for ~18 hours. eGFP expression was observed under fluorescence microscope (left, untreated; right, treated by 50 nM PMA).

Notes

To generate the AP1 eGFP reporter stable cell line, remove the growth medium 48 hours after transduction and replace it with fresh growth medium containing the appropriate amount of G418 for antibiotic selection of transduced cells. For information about determining the appropriate concentration of selection antibiotic, visit https://bpsbioscience.com/cell-line-faq in our Resources section (Q: what is a kill curve?)

References

1. Kyriakis J.M., et al. *Nature*. 1994; **369**: 156–160.
2. Lee W., et al. *Nature*. **325** (6102): 368–72.

Troubleshooting Guide

Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
AP1 Reporter (Luc) - HEK293 Cell line	60405	2 vials
AP1 Reporter Kit (JNK Signaling Pathway)	60612	500 reactions
AP1 Luciferase Reporter Lentivirus	79823	500 µl x 2
Negative Control eGFP Reporter Lentivirus	79927	500 µl x 2
Bald Lentiviral Pseudovirion (eGFP Reporter)	79987	500 µl x 2