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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The GAS Luciferase Reporter Lentiviruses are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to transduce almost all types of mammalian cells, including primary and non-dividing cells. The particles contain a firefly luciferase gene driven by three copies of the interferon gamma (IFN-γ) activated sites (GAS) located upstream of the minimal TATA promoter (Figure 1) and a puromycin selection gene for the selection of stable clones. After transduction, the GAS-regulated gene expression in the target cells can be monitored by measuring the luciferase activity.

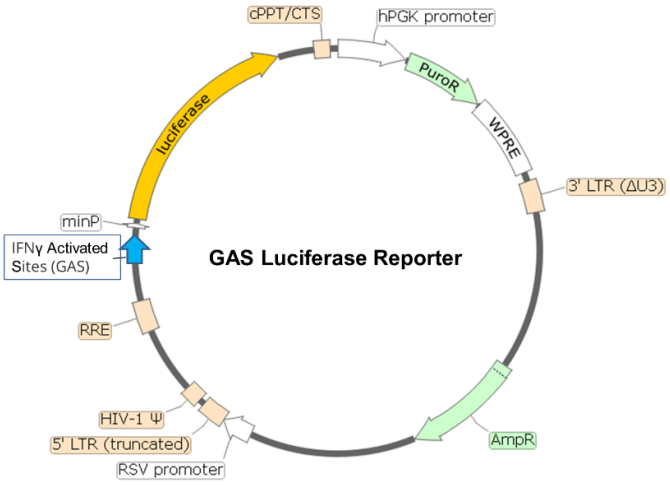

Figure 1. Schematic of the lenti-vector used to generate the GAS luciferase reporter lentivirus.

Background

Interferon gamma (IFN-γ) is a pleiotropic cytokine with anti-viral, anti-tumor, and immune-modulatory functions. Cellular responses to IFN-γ are activated through its interaction with a heterodimeric receptor consisting of two subunits, Interferon gamma receptor 1 (IFNGR1) and Interferon gamma receptor 2 (IFNGR2), associated with kinases JAK1 and JAK2, respectively. Upon binding to this receptor, IFN-γ triggers JAK/STAT signaling. The activated STAT1 homodimers translocate to the nucleus where they bind interferon-gamma-activated sites (GAS) in the promoter of IFN-γ inducible genes, which stimulates IFN-γ-specific activation of gene transcription.

Application(s)

- Screen for activators or inhibitors of IFN-γ-induced signal transduction pathways.
- Generate GAS Luciferase Reporter stable cell line (puromycin resistant).

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 is provided with each shipment.

BPS Bioscience ● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

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 SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and after 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 this 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
HeLa	ATCC #CCL-2
Recombinant Human IFN-γ	PeproTech #300-02
Thaw Medium 1	BPS Bioscience #60187
96-well tissue culture, clear-bottom, white plate	Corning #3610
ONE-Step™ luciferase assay system	BPS Bioscience #60690
Luminometer	

Assay Protocol

The following protocol is a general guideline for transducing HeLa cells using the GAS luciferase reporter lentivirus. The optimal transduction conditions (e.g., MOI, concentration of polybrene, time of assay development) 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 select the cells stably expressing the reporter gene with puromycin prior to carrying out the reporter assays.

1. Day 1: Seed HeLa 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 1 (BPS Bioscience #60187).

   To each well, add 1 µl of GAS luciferase reporter lentivirus. *Optional: Add polybrene to each well to 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.

   Add 100 µl of Thaw medium 1 supplemented with 1 ng/ml of IFN-γ to stimulated wells.

Add 100 µl of Thaw medium 1 to the control untreated wells (to determine the luminescence from the transduced HeLa cells not stimulated with IFN-γ).

Add 100 µl of Thaw medium 1 to cell-free control wells (for determine the background luminescence).

Incubate the plate at 37°C with 5% CO_2 overnight.

3. Day 4: Perform the ONE-Step™ Luciferase assay (BPS Bioscience #60690) as per recommended protocol (100 µl/well). Incubate the plate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer.

Validation Data

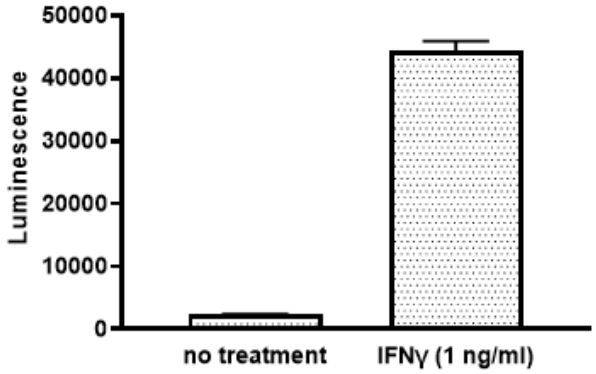

Figure 2. Activation of GAS luciferase reporter activity by IFN-γ in HeLa cells.
Approximately 8,000 HeLa cells/well were transduced with 20,000 TU/well GAS Luciferase Reporter Lentivirus. After 48 hours of transduction, the medium was changed to Thaw Medium 1 or to Thaw Medium 1 containing IFN-γ (1 ng/ml), and the plate was incubated at 37°C with 5% CO_2 overnight. Results are shown as the raw luminescence reading.

Notes

1. To generate a GAS luciferase reporter stable cell line, remove the growth medium 48 hours after transduction and replace it with fresh growth medium containing the appropriate amount of puromycin for antibiotic selection of transduced cells.
2. The following Lentivirus Reporter Controls are available from BPS Bioscience to meet your experimental needs:
    a. Negative Control Luciferase Lentivirus (BPS Bioscience #79578): Ready-to-transduce lentiviral particles expressing firefly luciferase under the control of a minimal promoter. The negative control is important to establish the specificity of any treatments and to determine the background reporter activity.
    b. Renilla Luciferase Lentivirus (BPS Bioscience #79565): Ready-to-transduce lentiviral particles expressing Renilla luciferase under the CMV promoter. The Renilla Luciferase lentivirus can serve as an internal control to overcome sample-to-sample variability when performing dual-luciferase reporter assays.
    c. Firefly Luciferase Lentivirus (BPS Bioscience #79692-G, #79692-H, #79692-P): Ready-to-transduce lentiviral particles expressing firefly luciferase under the CMV promoter. It serves as a positive control for transduction optimization studies.

Reading Luminescence

Luminescence is the emission of light resulting from a chemical reaction. The detection of luminescence requires no wavelength selection because the method used is emission photometry and not emission spectrophotometry.

To properly read luminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay.

References

1. Decker T, Kovarik P, Meinke A. (1997) GAS elements: a few nucleotides with a major impact on cytokine-induced gene expression. J Interferon Cytokine Res. 17(3): 121- 34.
2. Darnell J, Kerr IM, Stark GR. (1994) Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science 264: 1415-1421.

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*
Negative Control Luciferase Lentivirus	79578	500 µl x 2
Firefly Luciferase Lentivirus (G418, Hygromycin and Puromycin)	79692	500 µl x 2
Renilla Luciferase Lentivirus (G418 or Puromycin)	79565	500 µl x 2
ISRE Luciferase Reporter Lentivirus (JAK/STAT Signaling Pathway)	79824	500 µl x 2
STAT3 Luciferase Reporter Lentivirus	79744	500 µl x 2
STAT5 Luciferase Reporter Lentivirus	79745	500 µl x 2