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Produktinformation

- Forschungsprodukte & Biochemikalien
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- Diagnostik & molekulare Diagnostik
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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The p53 Luciferase Reporter Lentiviruses are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to transduce most types of mammalian cells, including primary and non-dividing cells. The particles contain a firefly luciferase gene driven by p53 response elements located upstream of the minimal TATA promoter (Figure 1) and an antibiotic selection gene (puromycin) for the selection of stable clones. After transduction, p53-regulated gene expression in the target cells can be monitored by measuring the luciferase activity.

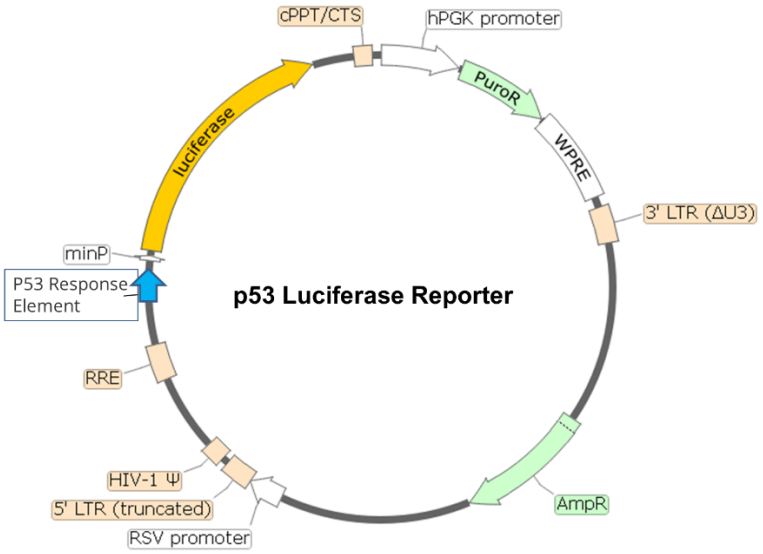

Figure 1. Schematic of the lenti-vector used to generate the p53 luciferase reporter lentivirus.

Application

- Screen for activators or inhibitors of p53 signaling pathway
- Generate a stable p53 luciferase reporter cell line (puromycin resistant) following puromycin selection and limiting dilution cloning

Background

p53 is a transcription factor and tumor suppressor very frequently mutated in human cancer and often termed "guardian of the genome". Activated by DNA damage, oxidative stress, or deregulated oncogene expression, p53 binds to a specific site in the promoter region of target genes and leads to the transcriptional activation of downstream genes involved in DNA repair, cell cycle arrest, senescence, and apoptosis. Inactivation of p53 promotes genome instability and directly contributes to cell transformation.

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.

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 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
HCT116	ATCC #CCL-247
Thaw Medium 7	BPS Bioscience #60185
Assay Medium 7A	BPS Bioscience #78673
Mitomycin c	Sigma #M4287-2MG
Nutlin-3	Sigma #N6287-1MG
Doxorubicin	Sigma #D1515
96-well tissue culture, clear-bottom, white plate	Corning #3610
One-Step Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Media Formulations

For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. Other preparations or formulations of media may result in suboptimal performance.

Media Required for the Proposed Assay

Thaw Medium 7 (BPS Bioscience #60185):

McCoy's 5A medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Assay Medium 7A (BPS Bioscience #78673):

McCoy's 5A medium supplemented with 0.5% charcoal stripped FBS.

Assay Protocol

The following protocol is a general guideline for transducing HCT116 cells using the p53 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 HCT116 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 7 (BPS Bioscience #60185).

   To each well, add 2 µl of p53 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 Assay Medium 7A containing the tested compounds to stimulated wells.

   Add 100 µl of Assay Medium 7A to the positive control untreated wells (to determine the luminescence from the transduced HCT116 cells).

   Add 100 µl of Assay Medium 7A 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 the recommended protocol (100 µl/well). Incubate the plate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer.

Validation Data

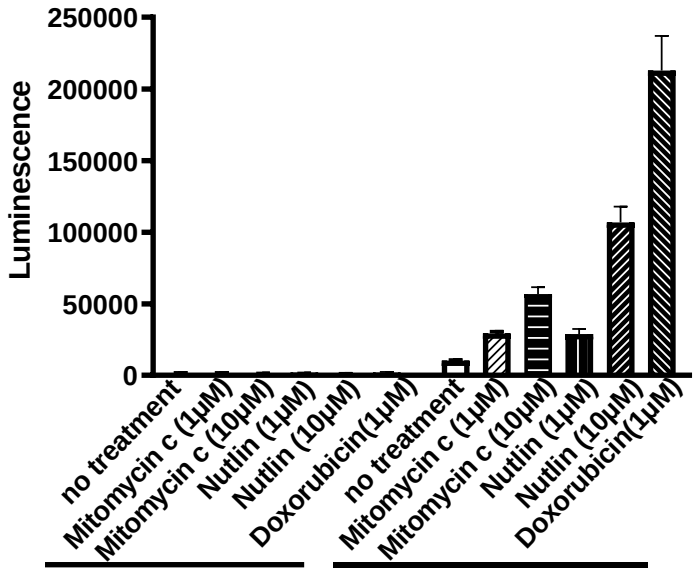

Figure 2. Activation of p53 luciferase reporter activity in HCT116 cells.
Approximately 8,000 HCT116 cells/well were transduced with 40,000 TU/well p53 Luciferase Reporter Lentivirus. After 48 hours of transduction, the medium was changed to Assay Medium 7A containing tested compounds, and the plate was incubated at 37°C with 5% CO_2 overnight. Results are shown as the raw luminescence reading. The Negative Control Luciferase Lentivirus (BPS Bioscience #79578) was performed in parallel as control.

Notes

1. To generate a p53 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. To determine the concentration of puromycin needed for your cell line, perform a kill curve (https://bpsbioscience.com/cell-line-faq; what is a kill curve?).
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. This 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.

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*
p53, GST-Tag Recombinant	40511	20 µg
Myc Reporter (Luc) HCT116 Recombinant Cell Line (Myc Signaling Pathway)	60520	2 vials
MDM2, GST-tag Recombinant	100409	20 µg
MDM2 TR-FRET Assay Kit	79773	384 reactions