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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

Data Sheet

Secreted Gaussia Luciferase Lentivirus (CMV Promoter) **Catalog #: 79892-C**

Product Description

The Secreted Gaussia Luciferase Lentivirus 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 constitutively express secreted Gaussia luciferase under a CMV promoter (Figure 1).

Application

1. Useful as a sensitive reporter to monitor important biological processes;
2. Generation of stable cell line expressing secreted Gaussia luciferase with puromycin selection.

Formulation

The lentiviruses were produced from HEK293T cells in medium containing 90% DMEM + 10% FBS.

Titer

Two vials (500 μ l x 2) of Secreted Gaussia Luciferase Lentivirus at a titer $\geq 5 \times 10^6$ TU/ml. The titer will vary with each lot; the exact value is provided with each shipment.

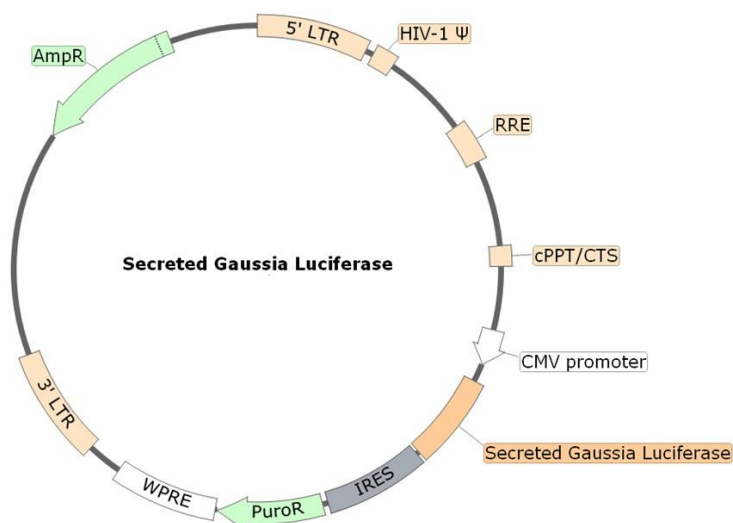


Figure 1. Schematic of the lenti-vector used to generate the Secreted Gaussia Luciferase Lentivirus

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6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
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Storage

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

Biosafety

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. Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.

Materials Required but Not Supplied

- CHO-K1 cells (ATCC #CCL-61)
- CHO growth medium or use BPS Thaw Medium 3 (BPS Bioscience #60186)
- Polybrene (Millipore, #TR-1003-G)
- 96-well tissue culture treated white clear-bottom assay plate (Corning, #3610)
- Coelenterazine native (NanoLight Technology, #303-500): Make a 2 mM stock in Ethanol
- CHO lysis buffer: PBS+1% Triton X-100
- Luminometer

Assay Protocol

The following protocol is a general guideline for transducing CHO-K1 cells using Secreted Gaussia Luciferase 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 Gaussia luciferase 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 Gaussia luciferase with puromycin prior to carrying out the reporter assays.

1. Day 1: Harvest CHO-K1 cells from culture and seed cells at a density of 5,000-10,000 cells per well into white opaque 96-well microplate in 50 µl of CHO growth medium. Incubate cells at 37°C with 5% CO₂ overnight.
2. Day 2: To each well add 5 µl of Secreted Gaussia Luciferase Lentivirus or Expression Negative Control Lentivirus (BPS Bioscience #79902-P). 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% CO₂ for 18-24 hours.
Alternatively, seeding cells and the transduction can be performed on the same day.
3. Day 3: Remove the medium containing the lentivirus from the wells. Add 100 µl of fresh CHO growth medium to each well.

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4. On the morning of Day 5, transfer the medium from each well to a new well. Gently wash the cells once with were washed gently with PBS once, and then lyse in 50 μ l of CHO lysis buffer (PBS+1% TritonX-100) for 15 minutes.
5. Dilute Coelenterazine stock to 25 μ M with PBS. Transfer 10 μ l of medium or cell lysate into a new well. Add 40 μ l of 25 μ M Coelenterazine into each well, and measure the luminescence IMMEDIATELY using a luminometer.

Important Notes:

To generate a secreted Gaussia luciferase stable cell line, on day 4 remove CHO growth medium and replace it with fresh growth medium containing the appropriate amount of puromycin for antibiotic selection of transduced cells.

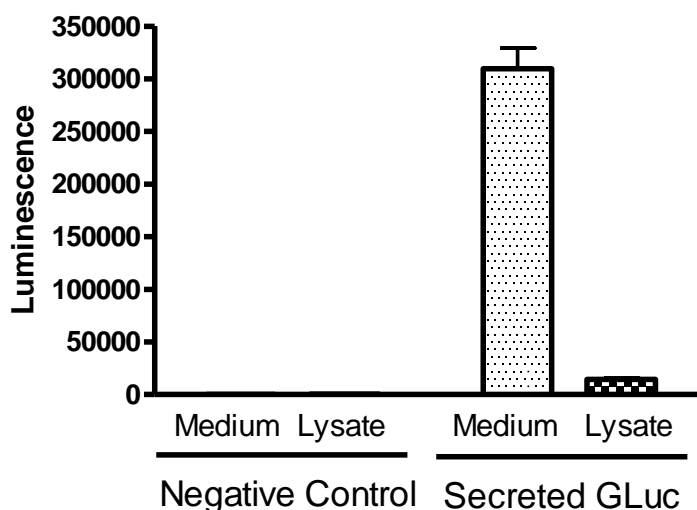


Figure 2. Gaussia luciferase activity in CHO-K1 cells transduced with Secreted Gaussia Luciferase Lentivirus. 10,000 CHO cells/well were transduced with 50,000 TU/well Secreted Gaussia Luciferase Lentivirus or Expression Negative Control Lentivirus in CHO growth medium. After 18 hours of transduction, medium was changed to fresh CHO growth medium. After 66 hours of transduction, cells were lysed and both the medium and cell lysate were assayed for Gaussia luciferase activity.

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Related Products

<u>Product</u>	<u>Cat. #</u>	<u>Size</u>
NFκB Luciferase Reporter Lentivirus	79564	500 µl x2
CRE Luciferase Reporter Lentivirus	79580	500 µl x2
NFAT Luciferase Reporter Lentivirus	79579	500 µl x2
STAT3 Luciferase Reporter Lentivirus	79744	500 µl x2
STAT5 Luciferase Reporter Lentivirus	79745	500 µl x2
TCF/LEF Luciferase Reporter Lentivirus	79787	500 µl x2
ISRE Luciferase Reporter Lentivirus	79824	500 µl x2
IL-2 Promoter Luciferase Reporter Lentivirus	79825	500 µl x2
IL-8 Promoter Luciferase Reporter Lentivirus	79827	500 µl x2
AP-1 Luciferase Reporter Lentivirus	79823	500 µl x2
SBE Luciferase Reporter Lentivirus	79806	500 µl x2
TEAD Luciferase Reporter Lentivirus	79833	500 µl x2
ARE Luciferase Reporter Lentivirus	79869	500 µl x2
Negative Control Lentivirus	79578	500 µl x2
Renilla Luciferase (Rluc) Lentivirus	79565	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (G418)	79692-G	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (Hygromycin)	79692-H	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (Puromycin)	79692-P	500 µl x2
FcGR11A Lentivirus	79876	500 µl x2
FcGR11B Lentivirus	79877	500 µl x2
FcER1G Lentivirus	79878	500 µl x2
Expression negative Control Lentivirus	79902	500 µl x2
TCR Activator Lentivirus	79894	500 µl x2
Non-Secreted Gaussia Luciferase Lentivirus	79893	500 µl x2

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