EV-Luminite™ EV Labeling System

Cat # EVLT-XXX-X

User Manual

Storage:

Store all plasmids at -20C, all viruses and EVs at -80°C and cell lines in liquid nitrogen.

Version 1
11/2/2023

Contents

Product Description

EV-Luminite™ is an innovative product designed to revolutionize the field of Extracellular Vesicles (EVs) research and analysis. With its advanced technology and unique approach, EV-Luminite™ enables the precise quantification of EV subpopulation secretion and delivery by harnessing a luminescent signal. This cutting-edge product allows researchers and scientists to shed light on the dynamics and magnitude of EV release, transfer, and EV subpopulation function, opening new avenues for understanding cellular communication and therapeutic applications.

SBI's EV-Luminite™ offers:

• Accurate Luminous Quantification

• High Sensitivity and Reliability

• Stable Lenti-based System

• Enhanced Visibility into EV Subpopulation Processes

• Customizable Hight-Content Screening (HCS) Applications

• Advanced Research and Therapeutic Potential

Unlock the secrets hidden within extracellular vesicles and elevate your research to new heights with EV-Luminte™. Join the revolution in cellular communication and explore the limitless possibilities this innovative tool offers. Illuminate the future of EV world with EV-Luminte™.

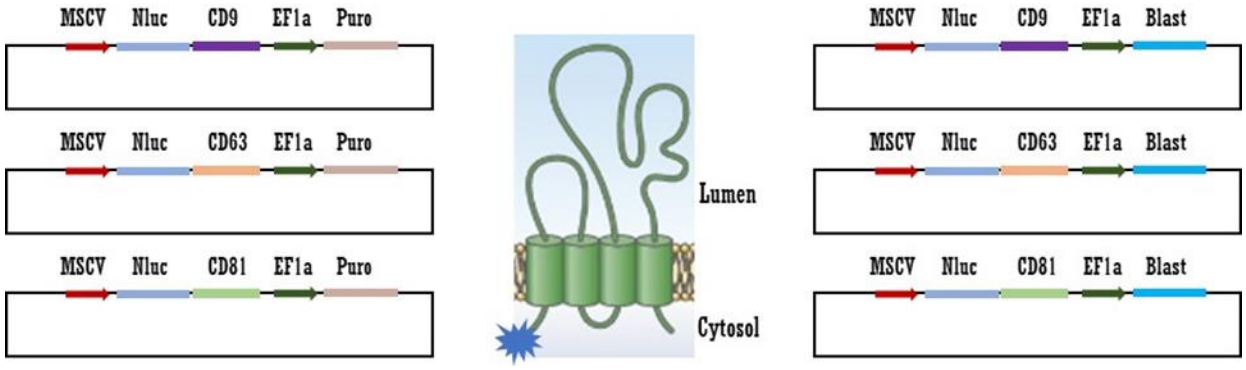


Figure 1. EV-Luminite™ constructs. *Nluc enzyme was cloned into CD9, CD63 or CD81 encoding lenti construct at the N-terminal position with either puromycin or blasticidin resistant. Middle: Scheme of the topology of a tetraspanin (CD9, CD63 or CD81) with N-terminal Nluc-tag.*

List of Components

Table 1. Components of GEQM8XXA-1 or KIT products

Catalog Number	Product Name	Quantity
EVLT-CD9-PA-P	EV-Luminite™ pCDH-MSCV-NLuc-CD9-EF1-Puro Expression Lentivector	10 µg
EVLT-CD63-PA-P	EV-Luminite™ pCDH-MSCV-NLuc-CD63- EF1-Puro Expression Lentivector	10 µg
EVLT-CD81-PA-P	EV-Luminite™ pCDH-MSCV-NLuc-CD81- EF1-Puro Expression Lentivector	10 µg
EVLT-CD9-PA-B	EV-Luminite™ pCDH-MSCV-NLuc-CD9- EF1-Blast Expression Lentivector	10 µg
EVLT-CD63-PA-B	EV-Luminite™ pCDH-MSCV-NLuc-CD63- EF1-Blast Expression Lentivector	10 µg
EVLT-CD81-PA-B	EV-Luminite™ pCDH-MSCV-NLuc-CD81- EF1-Blast Expression Lentivector	10 µg
EVLT-CD9-VA-P	EV-Luminite™ pCDH-MSCV-NLuc-CD9-EF1-Puro Expression Lentivirus	>1 x 10^6 IFUs
EVLT-CD63-VA-P	EV-Luminite™ pCDH-MSCV-NLuc-CD63-EF1-Puro Expression Lentivirus	>1 x 10^6 IFUs
EVLT-CD81-VA-P	EV-Luminite™ pCDH-MSCV-NLuc-CD81-EF1-Puro Expression Lentivirus	>1 x 10^6 IFUs
EVLT-CD9-CL-1	EV-Luminite™ MSCV-NLuc-CD9 HEK293 puro resistant stable cell line	2 x 10^6 Cells
EVLT-CD63-CL-1	EV-Luminite™ MSCV-NLuc-CD63 HEK293 puro resistant stable cell line	2 x 10^6 Cells
EVLT-CD81-CL-1	EV-Luminite™ MSCV-NLuc-CD81 HEK293 puro resistant stable cell line	2 x 10^6 Cells
EVLT-CD9-EXOP-1	Exosomes from EV-Luminite™ pCDH-MSCV-NLuc-CD9 HEK293 puro resistant stable cell line	50 µg
EVLT-CD63-EXOP-1	Exosomes from EV-Luminite™ pCDH-MSCV-NLuc-CD63 HEK293 puro resistant stable cell line	50 µg
EVLT-CD81-EXOP-1	Exosomes from EV-Luminite™ pCDH-MSCV-NLuc-CD81 HEK293 puro resistant stable cell line	50 µg

Additional Required and Optional Equipment Not Included in Kit

1. Plasmid propagation: RecA- and EndA- E.coli competent cell (We use OneShot OmniMax 2T1R in house), Carbenecillin
2. pPACH1 HIV Lentiviral Packaging Kit (Cat# LV500A-1, LV510A-1)
3. PureFection Transfection reagent (Cat# LV750A-1, LV750A-5)
4. HEK293 TN producer cell line (Cat# LV900A-1)
5. PEG-It Lentivirus concentration Reagent (Cat# LV810A-1, LV825A-1)
6. TransDux Lentivirus Transduction Enhancer (Cat# LV850A-1)
7. UltraRapid Global Titering Kit (Cat# LV961A-1)
8. Exosome-depleted FBS (Cat# EXO-FBSHI-50A-1, EXO-FBSHI-50A-1)
9. Nano-Glo Luciferase Assay System, Promega
10. ExoQuick-TC Exosome Precipitation Reagent (Cat# EXOTC10A-1, EXOTC50A-1)
11. EV-Guard™ EV storage buffer (EXSBA-1 or EXSBA-10)
12. Any Luminometer

How it works

EV-Luminte™ works by utilizing Nano luciferase detection system to quantify the production/secretion of Extracellular Vesicles (EVs), evaluate EV delivery and even tracing the cellular communication of EV. Here's an overview of how it works:

- Transduce your target cell with EV-Luminte™ lentivirus
- Generate EV-Luminite™ stable cell line
- Isolation EV from supernatant of EV-Luminte™ stable cell line
- Quantify Nano Luminescent signal for EV production/secretion
- Incubate EV released from EV-Luminte™ stable cell line with recipient cells
- Assess Nano luminescent signal in recipient cells or animal for EV delivery efficiency or dynamic biodistribution

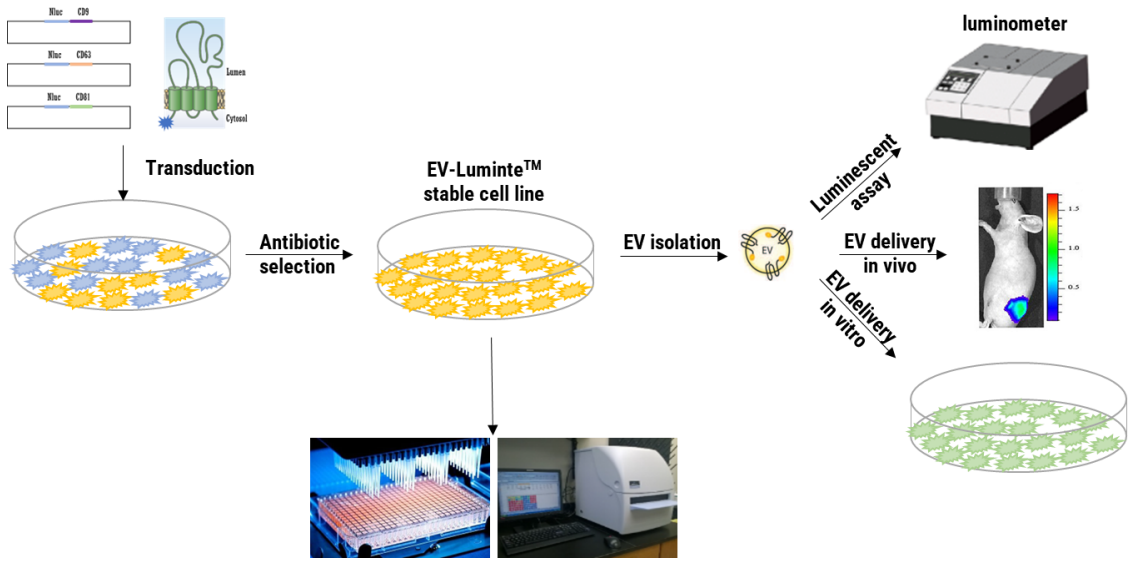


Figure2: Brighten up your EV research with EV-Luminite™. *Scheme of how EV-Luminite™ can help with your EV research. Transduce your target cells with EV- Luminte™ lentivirus to generate EV-Luminite™ stable cell line. EV released from EV-Luminte™ stable cell line can be qualified by nano luciferase assay, can be delivered to recipient cells to assess EV delivery/uptaken or can be delivered in mice to study EV dynamic biodistribution. The EV-Luminite™ stable cell line can be used for any customized high-content screening, such as screening for compounds that can increase EV secretion, et al.*

Protocol

A. General Comments

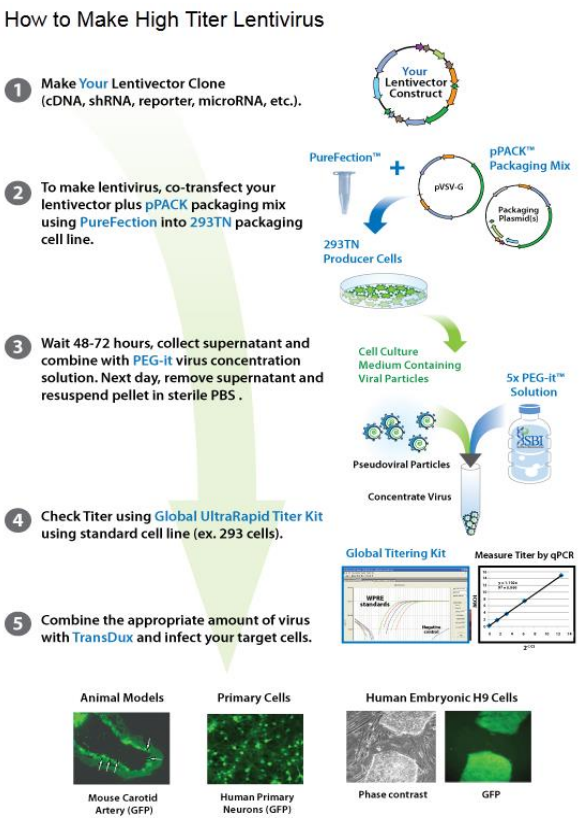
We recommend propagation of the lentivector plasmids of EV-Luminite™ prior to starting the experiments. The plasmids can be transformed using standard conditions suitable in any RecA- and EndA- E.coli competent cell.

Cells with the EV-Luminite™ constructs should be grown on LB-Carbenicillin plates (50µg/ml). Incubate the plates at 37°C overnight. Colonies picked from the transformation can be grown at 30°C overnight in ~200ml of LB media containing carbenicillin. After overnight growth, plasmid DNA can be harvested from culture using an endotoxin-free DNA plasmid maxiprep kit.

For confirmation of the plasmid, we recommend performing restriction digestion analysis or direct sequencing to confirm integrity of the amplified plasmids.

B. EV-Luminite™ Lentiviral Particle Production

For researchers looking for sustained, long-term expression of the EV-Luminite™ constructs in their desired cell line, the EV-Luminite™ constructs can be transfected into HEK293T producer cells and packaged into pseudolentiviral particles for infection of a target cell line. The following schematic and the protocol that follows shows the lentiviral production process using the EV-Luminite™ lentiviral constructs.



Workflow for generating high-titer lentiviral particles

1. Transfection of EV-Luminite™ plasmids into HEK293T (or equivalent) producer cells

a) 18 - 24 hours prior to transfection, seed 7.0 – 8.0 x10^6 293T cells per 150mm cell culture plate in standard growth media w/o selection antibiotics. Cells should be ~80% confluent by next day.

b) During transfection day, mix 45 µl of pPACKH1 packaging plasmid mix as provided in the LentiStarter 2.0 Kit and 4.5 µg of EV-Luminite™ lentivector in 1.6 ml of serum-free DMEM by pipetting.

c) Add 55 µl PureFection into the same tube. Vortex for 10 seconds.

Note: If using other transfection reagents (e.g., Lipofectamine 2000) please follow suggested guidelines for 150mm plates.

d) Incubate mixture at room temperature for 15 minutes.

e) Add mixture drop-wise to the dish, and swirl to disperse evenly throughout the plates.

f) Change the medium ~12 hours (or next day) after transfection.

g) At 48 hours and 72 hours after transfection, collect the medium (which now contains pseudoviral particles) into a 50-ml sterile, capped conical centrifuge tube. Centrifuge at 3000 x g for 15 minutes at room temperature to pellet cell debris. Transfer the viral supernatant into a new tube.

Caution: You are working with infectious pseudoviral particles at this stage. Please follow the recommended guidelines for working with BSL-2 biosafety agents.

2. Concentration of Pseudoviral Particles

The PEG-it™ Virus Precipitation Solution in the LentiStarter 2.0 Kit provides a simple and highly effective means to concentrate lentiviral particles. PEG-it is a formulation of polyethylene glycol optimized for the precipitation of lentiviral-based particles. The PEG-it Virus Precipitation Solution is provided as a 5x solution.

1. Transfer supernatant containing virus to a sterile vessel and add 1 volume of cold PEG-it Virus Precipitation Solution (4°C) to every 4 volumes of virus supernatant.

(Example: 5ml PEG-it with 20ml viral supernatant).

2. Refrigerate overnight (at least 12 hours). Viral supernatants mixed with PEG-it Virus Precipitation Solution are stable for up to 4-5 days at 4°C.

3. Centrifuge supernatant/PEG-it mixture at 1500 × g for 30 minutes at 4°C. After centrifugation, the virus particles may appear as a beige or white pellet at the bottom of the vessel.

4. Discard the supernatant into a suitable biohazard waste container. Spin down residual PEG-it solution by centrifugation at 1500 × g for 5 minutes. Remove all traces of fluid by aspiration, taking great care not to disturb the precipitated lentiviral particles in pellet.

5. Resuspend lentiviral pellets in 1/500 to 1/1000 of original volume of pooled virus supernatant using cold, sterile Phosphate Buffered Saline (PBS) or DMEM containing 25mM HEPES buffer at 4°C.

For example, if you performed 2 collections from 2 x 150mm plates (20ml per plate), this would be approximately 80ml of media. You would resuspend the resulting pellet in 80-160 µl of 1X PBS or DMEM.

6. Aliquot in cryogenic vials and store at -80°C until ready for use.

7. The resulting pseudoviral particles can be accurately titered using SBI's UltraRapid Global Titering Kit (Cat #LV961A-1) **https://www.systembio.com/global-ultrarapid-lentiviral-titering-kit**

C. Transduction of EV-Luminite™ Pseudoviral Particles into Target Cells

The following protocol can be utilized for delivery of virus to your target cells. The following protocol is for infection of target cells in a single well of a 24-well plate – if using larger vessels please scale up reagents accordingly.

Day 1

1. Plate 75,000 cells per well into a single well of a 24-well plate in cell culture medium. Make sure that cells are well-dispersed and are not clumped together. Include wells for negative (non-infected) cells.

Note: If infecting target cells for the first time or an optimal MOI is not known, please titrate virus at varying

MOIs (1, 5, 10 and 20, etc.) to optimize transduction using a positive control virus with a fluorescent marker such as SBI's pre-packaged positive transduction control (Cat #CD511VB-1).

Day 2

2. Cells should be between 70-80% confluent. Aspirate medium from cells.

3. Combine culture medium with TransDux to a 1X final concentration. For example, add 2.5 µl of TransDux to 500 µl culture medium and then transfer to each well.

4. Add EV-Luminite™ virus at desired MOI to each well and swirl to mix, for negative control wells only add media/viral transduction reagent.

Day 3

5. Aspirate off medium and add complete growth medium to cells.

Day 5

6. Virus should be integrated into the host cell genome by this time, and should be expressing the EV-Luminite™ construct.

D. Generation of EV-Luminite™ stable cell lines

Three days post-infection, begin puromycin or blasticidin selection for the generation of stable cell line. For 293T cells, the recommended concentration of puromycin is 0.5-1 µg/ml and the recommended concentration of blasticidin is 5-8 µg/ml.

Tech Note:

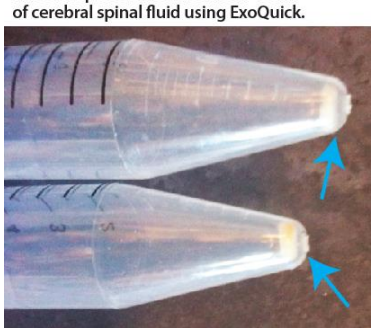
The effective working antibiotic concentration for a target cell line can be determined by establishing a kill-curve on untransfected cells. For example, the concentration of puromycin (typical working range of 0.5 µg-5 µg/ml) that kills >90% of cells after 48hours of selection is the correct dose for the cells being selected.

E. EV isolation from EV-Luminite™ stable cells

We recommend culturing the EV-Luminite™ stable cells in Exo-FBS/Exo-FBSHI media for 2-3 days (or when it reaches ~80% confluency) prior to collection of EV to ensure sufficient EVs are isolated. This will require optimizing seeding densities for a given cell line to meet these conditions. EV released from EV-Luminite™ stable cell line can be isolated using ExoQuick-TC (EXOTC10A-1 or EXOTC50A-1).

1. Collect culture medium and centrifuge at 3000 × g for 15 minutes to remove cells and cell debris.
2. Transfer supernatant to a sterile vessel and add the appropriate volume of ExoQuick-TC to the culture medium (1:5). Mix well by inverting or flicking the tube.
3. Refrigerate overnight (at least 12 hours) at +4°C. The tubes should not be rotated or mixed during the incubation period and should remain upright.
4. Centrifuge ExoQuick-TC/culture mixture at 1500 × g for 30 minutes. Centrifugation may be performed at either room temperature or +4°C with similar results. After centrifugation, the EV may appear as a beige or white pellet at the bottom of the vessel. For some serum free culture medium, you may not see the pellet which is normal as well.

Exosome pellets obtained from 10 ml of cerebral spinal fluid using ExoQuick.



5. Aspirate supernatant. Spin down residual ExoQuick-TC solution by centrifugation at 1500 × g for 5 minutes. Remove all traces of fluid by aspiration, taking great care not to disturb the precipitated EV in pellet.
6. Resuspend EV pellet in 100-500 µl using sterile 1X PBS, or specific buffer according to your downstream application. We recommend using EV-Guard™ EV storage buffer (EXSBA-1 or EXSBA-10) to store your EV samples for future use.

F. Luminescent activity of EV released from EV-Luminite™ stable cells

For EV isolated from EV-Luminite™ stable cells cultured in 10 cm dish, 500 µl of PBS or EV-Guard™ EV storage buffer was used to suspend EV pellet after ExoQuick-TC precipitation. EV protein concentration was measure by Nanodrop. 3 µl -5 µl of EV sample was added to 96-well plate for Nano luciferase assay. Nanoluc reagent was freshly reconstituted according to manufacturer's instructions (Nano-Glo Luciferase Assay System, Promega). Equal volume of Nanoluc substrate was mixed with EV sample and incubate at room temperature for 5 minutes. Then 100 µl of PBS was added to each well and ready for the plate reading. Nano luciferase activity can be further normalized by EV protein amount.

Per SEC study (E Grisard et al, J Extracell Vesicle 2022 Jul;11(7): e12242), ~70% of the nano luciferase activity comes from EV in the supernatant. Therefore, for high through put application, cell supernatant can be directly used for measuring EV secretion/production with Nano luciferase activity. For example, 40 µl of medium from 96-

well or 384 -well plate, can be transferred from cell plates to V-shaped 96-well or 384-well plates. Plates were then centrifuged at 350 g for 10 min at RT. 30 µl of centrifuged supernatant were then transferred to flat bottom plates. Nanoluc reagent was freshly reconstituted according to manufacturer's instructions (Nano-Glo Luciferase Assay System, Promega). 5 µl of reconstituted solution were added to each well using a Multichannel pipette. Plates were shaken for 5 minutes on an orbital shake prior to plate reading.

G. EV delivery of EV-Luminite™ stable cells to recipient cells

1. 250 µg EV from EV-Luminite™ stable cell line were added to naïve HEK293T recipient cells in culture in a 12-well plate format.
2. 24 hours after EV addition, recipient cells were trypsinized and washed twice with PBS.
3. 100 µl of passive lysis buffer were added to lyse the recipient cells.
4. 15-40 µl of cell lysate was used for measuring the nano luciferase activity by adding equal amount of Nano luciferase substrate using Nano-Glo luciferase assay

Supporting Data

EV-Luminite™ stable cell lines release EV with robust Nano luciferase activity

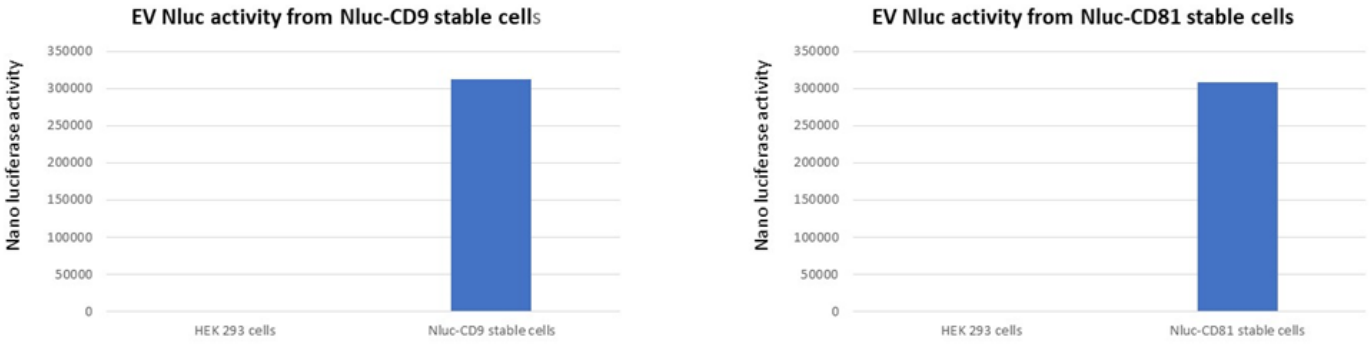


Figure 3. EV derived from EV-Luminite™ Stable cell lines reveal strong Nano Luciferase activity. *EV were isolated from Nluc-CD9 and Nluc-CD81 EV-Luminite™ Stable cell lines using ExoQuick-TC. Nano luciferase activity was measured using Nano-Glo Luciferase Assay.*

EV-Luminite™ offers accurate and sensitive quantification of EV

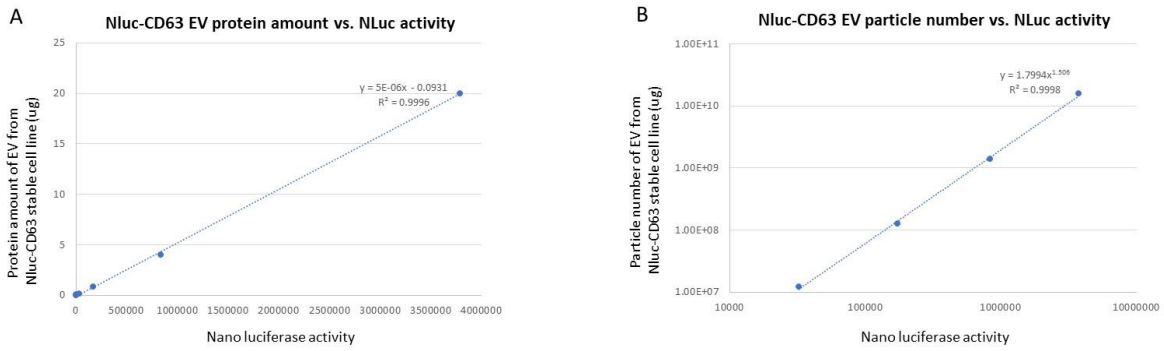


Figure 4. EV-Luminite™ allows accurate and sensitive quantification of EV. *EV from Nluc-CD63 EV-Luminite™ stable cell line was isolated using ExoQuick-TC. A) EV samples with different protein amount were used for measuring the Nano luciferase activity by Nano-Glo Luciferase Assay. B) EV samples with series dilution were used for measuring the Nano luciferase activity by Nano-Glo Luciferase Assay and particle number by NTA. Nano luciferase activity shows nice correlation with EV protein amount and particle number.*

EV-Luminite™ enables sensitive measurement of EV delivery to recipient cells

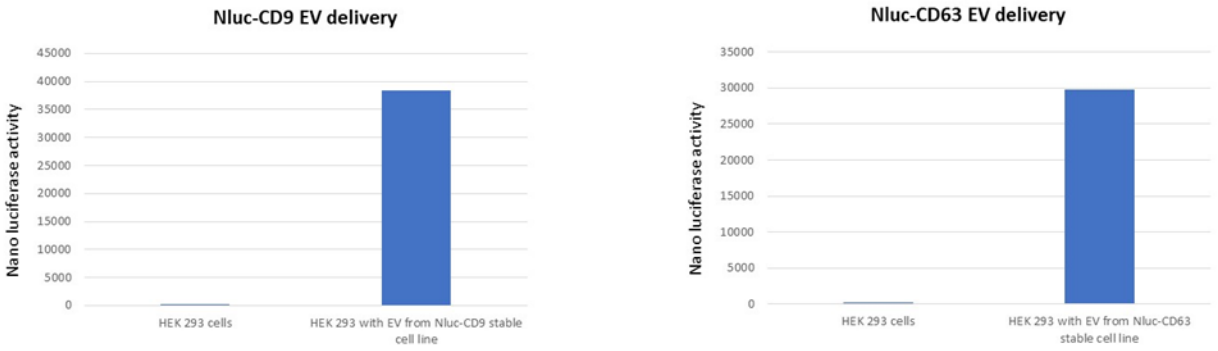


Figure 5. EV-Luminite™ provides a sensitive means of quantifying the delivery of EV to recipient cells. *EV were isolated from Nluc-CD9 and Nluc-CD63 EV-Luminite™ Stable cell lines using ExoQuick-TC. Isolated EV were then added to the culture medium of HEK 293 cells. 24-48 hours after EV delivery, HEK 293 cells were carefully washed then lysed. Nano luciferase activity of recipient cells was measured using Nano-Glo luciferase assay to assess the EV delivery.*

EV-Luminite™ empowers the evaluation of reagent impact on EV secretion/production

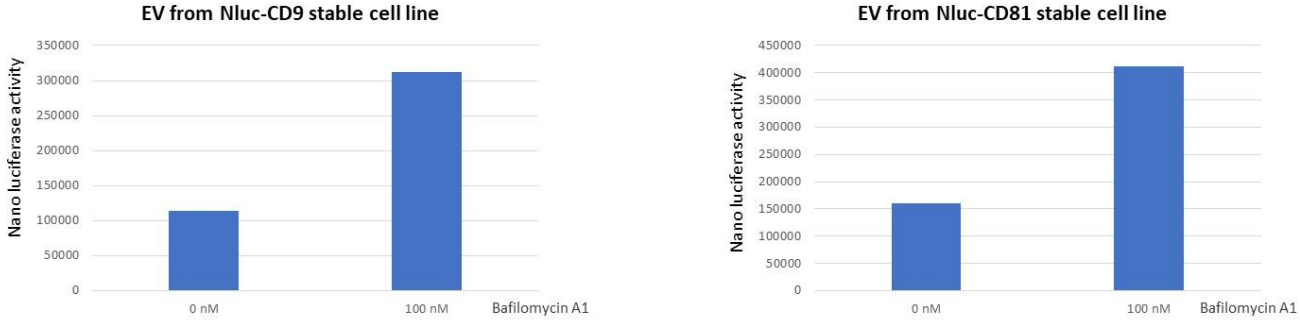


Figure 6. EV-Luminite™ serves as a useful tool to assess the effect of reagent on EV secretion/production. *Nluc-CD9 and Nluc-CD81 EV-Luminite™ stable cells were treated with DMSO vehicle control or Bafilomycin A1 (100nM) for over 5 hours. EVs were isolated using ExoQuick-TC. Nano luciferase activity of isolated EVs was measured using Nano-Glo luciferase assay normalized by EV protein amount.*

Technical Support

For more information about SBI products and to download manuals in PDF format, please visit our web site: **http://www.systembio.com**

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Licensing and Warranty Statement

Limited Use License

Use of the EV-Luminite™ EV Labeling System (*i.e.*, the "Product") is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:

- The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use.
- The Product may not be resold, modified for resale, or used to manufacture commercial products without prior written consent of SBI.
- This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

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Limited Warranty

SBI warrants that the Product meets the specifications described in this manual. If it is proven to the satisfaction of SBI that the Product fails to meet these specifications, SBI will replace the Product or provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to SBI within 30 days of receipt of the Product.

SBI's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. SBI's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. SBI does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

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