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Product Information

MISSION® 3'UTR Lenti GoClone™

SWITCHGEAR GENOMICS

Catalog Numbers: **HUTR00001-14350, HUTR001C-004C, CSTUTR**
Storage temperature –70 ˚C

TECHNICAL BULLETIN

Product Description
The 3' untranslated regions (3'UTRs) of mRNA transcripts control many aspects of post-transcriptional regulation of genes. 3'UTRs are involved in regulating transcript stability, translational efficiency, and localization in the cell. 3'UTRs are also the predominant target sequences for endogenous microRNAs (miRNA) or small interfering RNAs (siRNA).

The MISSION 3'UTR Lenti GoClone collection is a genome-wide collection of cloned human 3'UTRs. Each viral vector expresses an optimized luciferase reporter gene (RenSP) fused to a 3'UTR sequence.

The RenSP luciferase gene is one of the brightest reporter genes available and was specifically optimized for use with the LightSwitch Assay System to conduct highly reproducible and sensitive 3'UTR reporter assays.

The amphotropic lentivirus backbone allows for stable expression in a wide range of mammalian cell lines. After transduction with MISSION 3'UTR Lenti GoClones and selection for stable integration, miRNAs of interest may be introduced to determine whether or not they target the encoded 3'UTR via their effect on luciferase activity.

Unlike murine-based MMLV or MSCV retroviral systems, lentiviral-based systems such as MISSION 3'UTR Lenti GoClones, permit efficient infection and integration of constructs into differentiated and non-dividing cells, such as neurons and dendritic cells[1], overcoming low transfection and integration difficulties when using these cell lines. Self-inactivating replication incompetent viral particles are produced in packaging cells (HEK293T) by co-transfection with compatible packaging plasmids. [2-3]

In addition, the lentiviral transduction particles are pseudotyped with an envelope G glycoprotein from Vesicular Stomatitis Virus (VSV-G), allowing transduction of a wide variety of mammalian cells including primary and embryonic stem cells.[4] The lentiviral transduction particles are titered via a p24 antigen ELISA assay and pg/ml of p24 are then converted to transducing units per ml using a conversion factor.

Components/Reagents

Product	Quantity	Cat. No.
MISSION 3'UTR Lenti GoClone	0.2ml/ ≥10^5 TU	HUTR00001-14350, CSTUTR
MISSION 3'UTR Lenti GoClone-Controls	0.2ml/ ≥10^5 TU	HUTR001C-004C

The individual constructs are provided in Dulbecco's modified Eagle's Medium with 10% heat inactivated fetal bovine serum and penicillin-streptomycin. Volumes are 0.2 ml with ≥10^5 TU (transducing units). For vector backbone, see Figure 1 on Page 5.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Though the lentiviral transduction particles produced are replication incompetent, it is highly recommended that they be treated as **Risk Group Level 2 (RGL-2)** organisms.[5] Follow all published RGL-2 guidelines for handling waste decontamination.

Storage/Stability
All components are stable for at least six months after receipt when stored at –70 ˚C. Avoid repeated freeze/thaw cycles, which will severely reduce functional viral titer.

Reagents Required but not Provided

Transduction and Cell Culture
- Hexadimethrine Bromide, Catalog Number H9268
- Puromycin dihydrochloride, Ready Made Solution, 10mg/ml, Catalog Number P9620
- Growth medium optimized for the specific cell line

Reporter Assay Reagents
- MISSION LightSwitch Luciferase Assay Reagent, Catalog Number MLS0001 (100, 96-well assays)
- Plate Luminometer (SpectraMaxL or equivalent)

Knockdown by microRNA Mimic
- MISSION microRNA Mimic, Catalog Number HMI0001-0985
- Reagent for transfection, electroporation or nucleofection

Procedure for the Use of MISSION® 3'UTR Lenti GoClones

1 Week before transduction
When the appropriate concentration of puromycin for a specific cell type is unknown, perform a kill curve experiment, i.e., expose untransduced cells to increasing amounts of puromycin to determine lethal dose. Typically, puromycin concentrations ranging from 0.25–5 μg/ml are sufficient to kill most untransduced mammalian cell lines.

The time required for cells transduced with Mission 3′UTR Lenti GoClones to emerge from selection is often longer than that observed with transduction using other Mission lentiviral products. Therefore, we recommend using a lower concentration of puromycin than typically used with other Mission lentiviral products and extending the duration of puromycin selection. For example, a standard kill curve may indicate that 3 μg/ml puromycin results in 100% death in 5 days and 0.5 μg/ml puromycin results in 100% death in 10 days. In this example, we recommend using 0.5 μg/ml puromycin over a 10 day period. Optimal selection conditions will need to be determined for each cell line.

Puromycin titration (kill curve) should be performed when working with a new cell type.
1. Plate 1.6 x 10^4 cells into wells of a 96-well plate with 120 μL fresh media.
2. The next day add 0.5–10 μg/ml of puromycin to selected wells.
3. Examine viability every 2 days.
4. Culture for 3–14 days depending on the growth rate of the cell type and the length of time that cells would typically be under selection during a normal experimental protocol. Replace the media containing puromycin every 3 days. The minimum concentration of puromycin that causes complete cell death after the desired time should be used for that cell type and experiment.

Note: Excess puromycin can cause many undesired phenotypic responses in most cell types.

Day 1
Plate the mammalian cell line of choice in complete medium 24 hours prior to transduction at a level to give a 50-80% confluent culture for transduction.

Day 2
Thaw the lentiviral stock slowly on ice. Add hexadimethrine bromide (the chemical equivalent of Polybrene) to the cells at a final concentration of 8 μg/ml.

Note: Hexadimethrine bromide enhances transduction of most cell types. However, some cells, such as primary neurons, are sensitive to hexadimethrine bromide. Do not add hexadimethrine bromide when using sensitive cells.
Following the addition of hexadimethrine bromide, gently swirl the plate to mix. Add the appropriate amount of viral particles at a suitable multiplicity of infection (MOI) and swirl the plate gently to mix. Incubate the cell-viral particle mixture at 37 °C overnight.

Multiplicity of Infection (MOI) is the number of transducing lentiviral particles per cell. It is highly recommended that for each new cell type to be transduced, a range of MOI be tested.
To calculate MOI:
(total number of cells per well) x (desired MOI) = total transducing units needed (TU)

(total TU needed) / (TU/ml reported on C of A) = total ml of lentiviral particles to add to each well

Notes
a. When transducing a lentiviral construct into a cell line for the first time, it is recommended that a range of MOIs (0.5–20) be tested to find the optimal luminescence over background. Note that LentiGoClone constructs are significantly larger than those for other Mission lentiviral products, such as shRNAs. As a consequence, integration may be less efficient and therefore, higher MOIs and/or more cells may be needed with LentiGoClones. For example, $7.5x10^3$ HeLa cells were successfully transduced at MOI = 1 to 5 with LentiGoClones for MYC, ERBB2, ERBB3, and Nontarget Control 1 and 2 (3'UTRs = 0.7 to 1.4 kb), whereas those transduced at the same MOI in parallel with DICER1 LentiGoClone (3'UTR = 4 kb) all died during puromycin selection. Successful transduction with DICER1 was obtained with $1x10^5$ HeLa cells at MOI = 15.

b. When overnight incubation presents a toxicity concern, cells may be incubated for as little as 4 hours before changing the medium.

Day 3
Remove the viral particle-containing medium and replace it with fresh, pre-warmed complete culture medium.

Day 4
Perform one of the following based on whether the transduction experiment is transient or stable:

a. For transient expression experiments- Harvest the cells and assay for expression of the reporter gene.

b. For stable expression experiments- Release cells and split into two fresh wells, one for puromycin selection and the other as a backup culture in case cells have not adequately recovered. Seeding 80% for puromycin selection and 20% for backup culture has worked well. Proceed to day 5.

Day 5 and forward
Replace medium with fresh, puromycin-containing medium (as determined above from kill-curve) every 3–4 days until a population of puromycin-resistant cells has been established (generally, 10–12 days after selection).
Note: Infecting and selecting a pool of cells helps to minimize position effects due to random integration that may affect the expression levels in a clonal population of cells.

Knockdown of Reporter Gene Activity by Transfection of microRNA Mimic
For optimal transfection efficiency, screening a range of microRNA Mimic concentrations and cell densities is recommended. microRNA Mimic concentrations of 10–100 nM have been used successfully on a variety of cell lines but lower or higher concentrations may be necessary for specific applications. Volumes needed to obtain a final microRNA Mimic concentration of 10 nM in three different plate formats is shown in Technical Bulletin MI00100 for MISSION® microRNA Mimics.

A suggested protocol is shown as follows:

Micro-RNA Transfection Protocol
Note; MISSION microRNA Mimics NOT supplied with this product.
a. We suggest conducting at least 3 replicate transfections for each miRNA mimic or control. The tables below show suggested component amounts for a single-well transfection aliquot and for making a master mix for 3 replicate transfections (making 3.5 aliquots allows for some evaporation and pipetting error).
b. Dilute targeted miRNA mimics and non-targeting controls (NTCs) to 2 µm in RNase-free water.
c. Create two tubes for each miRNA mimic or NTC

Tube 1

Component	Per well (96-well format)	3.5x mix
miRNA mimic or NTC (2 µm)	varies	Varies
Opti-MEM (serum free)	to 10 µL	to 35 µL
TOTAL	10 µL	35 µL

Tube 2

Component	Per well (96-well format)	3.5x mix
Transfection reagent*	0.25 µL	0.88 µL
Opti-MEM (serum free)	9.75 µL	34.12 µL
TOTAL	10 µL	35 µL

*This protocol was optimized using Dharmafect 4 transfection reagents. For other transfection reagents and cell types, please refer to the manufacturer's protocols.

d. Incubate for five minutes at room temperature.
e. Mix tubes 1 and 2.
f. Incubate for 20 minutes at room temperature.
g. Combine the transfection reagent + miRNA mimic mixture with fresh media.

Component	Per well (96-well format)	3.5x mix
Mixture of tubes 1 & 2	20 µL	70 µL
Media (without puromycin)	80 µL	280 µL
TOTAL	100 µL	350 µL

h. Remove existing media from cells.
i. Add 100 µL of final transfection mixture to each of 3 replicate wells.
j. Incubate at 37C for 24 hours.

LightSwitch Protocol for Assaying Reporter Activity
Note: LightSwitch reagent NOT supplied with this product

Reagent preparation

1. Reconstitute 100X Substrate
   Add 100 µL Substrate solvent to tube of 100X Substrate (for 100 assay kit). Dissolve completely. Protect from light and minimize time at room temperature. 100X substrate may be stored at −20 °C and protected from light for 2-3 weeks. For best results, use freshly reconstituted substrate.

2. Prepare Assay Solution (for one 96-well plate)
   a. Thaw 10 mL bottle of Assay Buffer in room temperature water bath and add 100 µL of reconstituted 100X Substrate just prior to use. Mix well.
   b. Prepare Assay Solution (buffer + substrate mix) fresh for each use and use within 2-3 hours. To assay fewer wells, make up only what you need and store remaining substrate and buffer separately at −20 °C.

Assay reporter activity

1. Use a multi-channel pipettor to add 100 µL Assay Solution (buffer + substrate) directly to each sample well in a white 96-well plate.

   Bring sample plate to room temperature. Cells may be assayed in white 96-well TC plates directly from the incubator (100 µL media per well).

   Alternatively, plates with cells in media may be stored at −80 °C and thawed to room temperature for 45 minutes before assaying.

   If cells were grown in another plate or flask format, transfer samples to a white 96-well plate in 100 µL total volume (media or PBS).

2. Cover plate, protect from light, and incubate for 30 minutes at room temperature.

   If assaying more than one plate, stagger addition of assay solution so that each plate incubates for 30 minutes before reading.

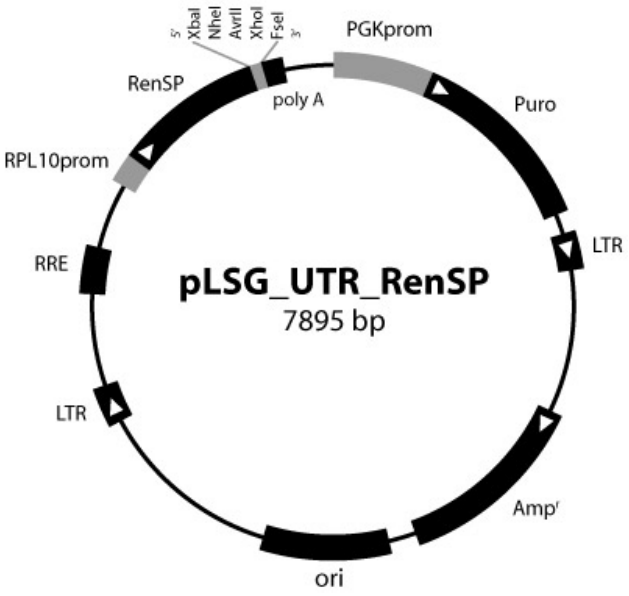
3. Read each well for 4 seconds in a plate luminometer (LMAXII384 or equivalent).

References

1. Stewart, S.A., *et al.*, Lenti-virus delivered stable gene silencing by RNAi in primary cells. *RNA*, 9, 493-501 (2003)
2. Zuffrey, R., *et al.*, Multiply attenuated lentiviral vector achieves efficient gene delivery *in vivo*. *Nat Biotechnol.* 15, 871-885 (1997)
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4. Burns, J.C., *et al.*, Vesicular Stomatitis Virus G Glycoprotein Pseudotyped Retroviral Vectors; Concentration to a Very High Titer and Efficient Gene Transfer into Mammalian and Nonmammalian Cells. *Proc. Natl. Acad. Sci. USA*, 90, 8033-8037 (1993).
5. NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines) 2002.

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Figure 1.
Features of
Lentiviral Plasmid Vector pLSG_UTR_RenSP



Name	Description
RPL10prom	Constitutive human RPL10 promoter
RenSP	Optimized *Renilla* luciferase gene
XbaI, NheI, AvrII, XhoI, FseI	Multiple Cloning Site for human 3'UTR insertion
PGKprom	Human phosphoglycerate kinase eukaryotic promoter
Puro	Puromycin resistance gene for mammalian selection
LTRs	Long terminal repeats
Amp^r	Ampicillin resistance gene for bacterial selection
ori	Origin of replication
RRE	Rev response element

Troubleshooting Guide

Problem	Cause	Solution
No or low levels of reporter gene expression due to low transduction efficiency	Hexadimethrine bromide not included during transduction	Transduce in the presence of hexadimethrine bromide.
	MOI is too low	Transduce at a higher MOI.
	Cells were harvested and assayed too soon after transduction	Reporter gene expression may be improved by placing cells under puromycin selection because untransduced cells will die.
	Not enough puromycin or puromycin inactive	Perform kill curve. Purchase new puromycin
	Viral stock stored incorrectly	Store stocks at –70 ˚C. Do not freeze thaw more than 3 times.
Cytotoxic effects observed after transduction	Hexadimethrine bromide was used during transduction	Be sure cells are not sensitive to hexadimethrine bromide. Omit hexadimethrine bromide during the transduction.
	Too much puromycin was used for selection	Determine the puromycin sensitivity of the cells by performing a kill curve and use the minimum concentration required to kill the untransduced cells.

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