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Product Information

MISSION® LentiExpress™ Human Kinases

Catalog Number **SHX001**
Storage Temperature –70 °C

TECHNICAL BULLETIN

Product Description
LentiExpress technology enables lentiviral shRNA screens in an easy, convenient and economical format. The technology employs controls and clones of the MISSION TRC shRNA libraries which are used in gene specific RNA interference (RNAi) in mammalian cells.

The MISSION product line includes lentiviral vector-based RNAi libraries against annotated mouse and human genes. shRNAs that generate siRNAs intracellularly are expressed from amphotropic lentivirus particles, allowing screening in a wide range of mammalian cell types. In these cells, MISSION shRNA clones permit rapid, cost efficient loss-of-function and genetic interaction screens.

MISSION LentiExpress Human Kinases is a pre-arrayed set of lentiviral particles allowing for rapid and effective whole kinome RNAi screening even in difficult to transfect cells. Protein kinases are among the largest and most well studied gene families. Protein phosphorylation plays an essential role in intercellular communication in eukaryotic organisms by mediating signal transduction during development, transcription, immune response, metabolism, apoptosis, and cell differentiation. Aberrant regulation of kinases plays a causal role in many diseases, and the study of these proteins and their functions will contribute to the discovery and development of new therapeutics.

MISSION LentiExpress Human Kinases contains ~3,200 different lentiviruses carrying shRNA sequences targeting ~501 human kinase genes, allowing for quick, high throughput loss-of-function screens. These kinase genes were chosen based on the 2002 paper by Manning, G., *et al.,*[1] and the RNAi Consortium. Each gene is represented by a clone set that consists of ~6 individual constructs, or clones, targeting different regions of the gene sequence. Therefore, a range of knockdown efficiencies can be expected, usually with 1 or more constructs from each gene set being >70%. Each shRNA construct has been cloned and sequence verified to ensure a match to the target gene.

The shRNA sequences are delivered to cells by replication incompetent lentiviral particles. Unlike murine-based MMLV or MSCV retroviral systems, lentiviral-based particles permit efficient infection and integration of the specific shRNA sequence into differentiated and non-dividing cells, such as neurons and dendritic cells[2]. Self-inactivating replication incompetent lentiviral particles are generated in producer cells (HEK 293T) by co-transfection with compatible packaging plasmids[3-4] (Catalog Number SHP001). 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.[5]

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. The conversion can be viewed at: www.sigma.com/titer.

Screens can be quickly carried out using LentiExpress by simply adding cells of interest into each well of the 96-well plate. Pre-arrayed transduction-ready particles allow for rapid lenti-shRNA based screening in a variety of mammalian cell types.

To achieve the best transduction efficiency, especially in hard to transduce cell lines, it is strongly recommended that the MISSION LentiExpress Optimization Plate, Catalog No. SHXC01, be used prior to screening. This product is intended for rapid determination of the optimal cell number (thus MOI) to use for LentiExpress assays.

Reagents/Components
MISSION LentiExpress Human Kinases consist of forty-one individual 96-well plates. In each plate, 88 wells contain 30 μL of shRNA Lentiviral Particles or controls and 8 wells contain 30 μL of media only. A CD contains detailed clone position, RefSeq, locus link, gene description, gene symbol, clone ID, and hairpin sequence.

Recommended controls for any shRNA experiment are described in the **Control Selection Table** that follows and are closely aligned with the controls suggested in the*Nature Cell Biology* editorial.[6]

Reagents and equipment required, but not provided
- Target cells in culture
- Complete growth media
- Hank's Balanced Salt Solution, Catalog No. H6648
- 1x Trypsin-EDTA solution, Catalog No. T3924
- 10% Bleach solution
- Hexadimethrine bromide, Catalog No. H9268
- Puromycin (10mg/ml solution), Catalog No. P9620
- Sterile pipette tips
- Centrifuge with swinging-plate rotor

Follow distributor's instructions for culturing and maintaining the target cell line. It is recommended to use the lowest passage # of cells possible for transduction experiments.

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 recommended that they be treated as **Risk Group Level 2 (RGL-2)** organisms in laboratory handling.[7] Follow all published RGL-2 guidelines for laboratory handling and waste decontamination.

Biosafety Features
The lack of generation of replication competent viral particles is an important safety feature of MISSION shRNA Lentiviral Particles. Safety features are summarized in Table 1. Users should consult and observe their own institutional guidelines when working with viral systems.

Table 1.

Biosafety Features of Third-Generation Lentiviral Particles

Feature	Result
Multi-plasmid approach	No single plasmid contains all the genes necessary to produce packaged lentivirus. Resultant particles are replication-incompetent.
SIN 3' LTR	Deletion in U3 portion of 3' LTR eliminates the promoter-enhancer region, avoids promoter interference issues, and further negates the possibility of viral replication, self-inactivating feature
Elimination of the majority of lentiviral genes (Δ vpr, vif, vpu and nef) [5]	Removes virulence genes which are not necessary for shRNA packaging systems
Chimeric 5' LTR	Reduces the chance of insertional oncogenesis, allows for tat independent transcription (tat has been implicated in various carcinomas).

Storage/Stability
After receiving immediately store the product at −70 °C. Avoid freeze/thaw cycles, as this will severely reduce transduction efficiency.

Representative Plate Layout

Lentiviral particles are provided in a pre-arrayed, ready-to-use format in 96-well plates. The MISSION LentiExpress Human Kinases consists of 41 plates, wherein each plate contains up to 80 different kinase-specific clones. Each well is pre-diluted to contain approximately 5,000 lentiviral particles in a single reaction volume of 30 µL. In addition to the kinase shRNA particles (**K**), each plate includes negative controls to monitor transduction efficiency:

	1	2	3	4	5	6	7	8	9	10	11	12
A	C	K	K	K	K	K	K	K	K	K	K	C
B	C	K	K	K	K	K	K	K	K	K	K	C
C	N	K	K	K	K	K	K	K	K	K	K	N
D	N	K	K	K	K	K	K	K	K	K	K	N
E	M	K	K	K	K	K	K	K	K	K	K	M
F	M	K	K	K	K	K	K	K	K	K	K	M
G	M	K	K	K	K	K	K	K	K	K	K	M
H	M	K	K	K	K	K	K	K	K	K	K	M

Wells A1, A12, B1 and B12 contain MISSION pLKO.1-puro Control Transduction Particles (**C**) Catalog No. SHC001V, as a negative control. These "empty vector" particles do not contain an shRNA insert and there is no evidence that they activate the RNAi pathway. These wells provide positive controls for efficient transduction since the puromycin resistance gene is expressed under control of the hPGK promoter (as in the kinase clones and other controls).

Wells C1, C12, D1 and D12 contain MISSION Non-Target shRNA Control Transduction Particles (**N**), Catalog No. SHC002V. This non-targeting shRNA is a useful negative control that will activate RISC and the RNAi pathway, but does not target any known human or mouse genes.

Wells E1, E12, F1, F12, G1, G12, H1 and H12 do not include shRNA lentiviral particles or control lentiviral particles, but contain 30 µL of media (**M**) and are intended for use as a negative control during the puromycin selection process.

Procedure Overview

Step	Description
1	Thaw the LentiExpress plates containing viral particles for 10 minutes at room temperature
2	Spin plates at 1000 rpm for 1 minute (if possible)
3	Remove sealer (important to thaw first)
4	Seed cells into LentiExpress plates with media containing hexadimethrine bromide
5	Change media to fresh media 24 hours post transduction
6	Add fresh media containing puromycin 48 hours post transduction
7	Grow cells to optimal confluency to perform assay of choice

Note: The appropriate concentration of puromycin for each cell type will vary. If the appropriate concentration for the desired cell type is unknown, a titration experiment, or kill curve, must be performed. Typically, 2–10 µg/ml are sufficient to kill most untransduced mammalian cell types.

Puromycin Titration:
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 medium.
2. The next day add 500–10,000 ng/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.

Procedure for each 96-well MISSION LentiExpress Human Kinase shRNA plate

Day 1: **Preparations**

1. Prepare container with 10% bleach solution for decontamination of pipette tips. Place container in a cell culture hood.
2. Thaw MISSION LentiExpress Human Kinase plates. Remove plates from freezer and place in the cell culture hood in a single layer. Allow plates to sit at room temperature until completely thawed, for no more than 30 minutes. Spin plate at 1000 rpm for 1 minute, then place back in the cell culture hood. Remove the plastic lid, securely hold the plate by its base, and remove and discard the seal. Replace the plastic lid on the plate until ready to add cells. Once all plates have been thawed and the seals removed immediately prepare cells and seed into plates.
   Note: It is important to thaw the plates before removing the sealer to prevent residual adhesive from being left on the plate.
3. Preparing cells for transduction
   a. Trypsinize cells and resuspend them in complete growth media.
   b. Transfer the cell suspension to a sterile 50 ml conical tube.
   c. Take a 50 μL sample of the cell suspension and mix with 50 μL of trypan blue. Count the cells and determine the cell density (cells/ml) using a Hemacytometer .
   d. Dilute cells to a final density of 14,500 cells/ml in a total of 11 ml per test plate using complete growth medium. This is for an approximate MOI of 5. Desired density and MOI will vary for different cell types rate and should be determined experimentally using the MISSION LentiExpress Optimization Plate.
   e. Add hexadimethrine bromide (the chemical equivalent of Polybrene) to the cell suspension to a concentration of 11.3 μg/ml (62.7μL of the 2 mg/ml stock per 11 ml of cell suspension). Once combined with the 30 μL in each well, this generates a final hexadimethrine bromide concentration of 8 μg/ml.
4. Transduction
   a. Mix the cells and then add 70 μL of cell suspension (~1000 cells) to each well in the 96-well plate (making sure the virus is already thawed before adding the cells). It is best to use a multi-channel pipette for this step, changing the tips after each addition.
   b. Decontaminate tips with 10% bleach solution and then dispose of them in the 10% bleach container.
   c. Place plate in an incubator at 37 °C in a humidified 5% CO_2 atmosphere for 18-20 hours.

Day 2: Adding fresh growth media to the cells

1. Prepare container with 10% bleach solution for decontamination of pipettes tips and media waste. Place it in a cell culture hood.
2. Remove 100 μL of medium from each well and replace with 100 μL of complete growth medium.
3. After adding fresh media to each row of the plate, change tips.
4. The waste medium and tips should be decontaminated in 10% bleach before disposal.
5. Place plate in an incubator at 37 °C in a humidified 5% CO_2 atmosphere for 24 hours.

Day 3 Selection

1. Prepare selection medium by transferring 50 ml of complete growth medium to a 50 ml conical tube.
2. Add the required amount of puromycin solution to the 50 ml of complete growth medium to the final desired concentration that was determined in the Puromycin Kill Curve.
3. After the 24 hour incubation remove the 100 μL of medium in all wells and replace with 100 μL of selection medium containing Puromycin.
4. Place the plate in 37 °C incubator with 5% CO_2.
5. Change media containing Puromycin every 2-3 days, replacing with additional selection media, until cells become 80-90% confluent.
6. Observe the cells in all the wells under microscope for growth confirmation.
   a. Wells A1, A12, B1 and B12 containing pLKO.1-puro Control Transduction Particles, and wells C1, C12, D1 and D12 containing Non-Target shRNA Control Transduction Particles, should contain viable cells.
   b. Wells E1, E12, F1, F12, G1, G12, H1and H12 contain no viral particles and therefore the cells should not withstand the puromycin selection.
7. Perform analysis of screening results according to assay of choice.

Assay Variations

1. Cell Density- seeding densities will vary depending on cell type. Use MISSION LentiExpress Optimization Plate (SHXC01) to determine optimal seeding density (MOI). Default density is 1,000 cells/ 70 μL so final cells/well is 1,000
2. Volume – total volume in wells can be adjusted depending on the specific cell type. Default volume is 100 μL
3. Polybrene concentration – cells can be sensitive to polybrene and a lower concentration may be needed. Default final concentration is 8 μg/ml.

References

1. Manning, G., *et al*., The Protein Kinase Complement of the Human Genome, *Science,* **298**, 1912-1934 (2002).
2. Stewart, S.A., *et al*., Lentivirus-delivered stable gene silencing by RNAi in primary cells. *RNA*, **9**, 493-501 (2003).
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5. 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).
6. Whither RNAi? *Nature Cell Biology*, **5**, 489-490 (2003).
7. NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines) 2002 (http://www4.od.nih.gov/oba)

Troubleshooting Guide

Problem	Cause	Solution
Low transduction efficiency	Cell density was not optimized: too low or too high	To determine optimal density and transduction efficiency of tested cells, first use MISSION LentiExpress Optimization Plate, Catalog No. SHXC01. When adding cells to the wells make sure they are mixed well. Keep mixing them periodically to insure the same amount of cells for each well.
	Cell conditions were not optimal	Make sure that cells are 90% viable. Use cells that have been subcultured for less than 20 passages.
	Cells are sensitive to Polybrene	Test cells for sensitivity to reagent by including control cells incubated with media containing Polybrene (8 μg/ml) If cells are sensitive to Polybrene omit the addition of this reagent. Cells will still be transduced but with lower efficiency.
	Polybrene was not included during transduction	Transduce in the presence of Polybrene when possible, as it enhances the transduction efficiency of most cell types.
	Puromycin concentration is not optimal for cell selection	For each new cell type used, it is recommended that a Puromycin Kill Curve be performed to determine the lowest concentration of puromycin needed to efficiently select transduced cells.
	Non-dividing cell type used	In general, non-dividing cell types are transduced with lentiviral constructs less efficiently than actively dividing cell lines. To achieve best transduction efficiency use LentiExpress Optimization Plate prior to screening.

Control Selection Table

Recommended Control	Objective
Negative Control: Untreated Cells	Untreated cells will monitor cell culture growth conditions and provide a reference point for comparing all other samples.
Negative Control: Transduction with empty pLKO.1 vector, containing no shRNA insert	MISSION pLKO.1-puro Control Transduction Particles, Catalog No. SHC001V. This vector does not contain an shRNA insert. Particles generated with this construct serve as a useful negative control during transduction. This controls transduction effects without subsequent activation of the RNAi pathway.
Negative Control: Transduction with Non-targeting shRNA	MISSION Non-Target shRNA Control Transduction Particles, Catalog No. SHC002V The vector contains an shRNA insert that does not target any human or mouse genes due to 5 base pair mismatches with known genes in those genomes. This controls for non-specific events during transduction and activation of the RNAi pathway.
Positive Control: Transduction with positive reporter vector	MISSION TurboGFP™ Control Transduction Particles, Catalog No. SHC003V This is a useful positive control for measuring transfection efficiency and optimizing transduction. The TurboGFP Control vector consists of the lentiviral backbone vector, pLKO.1-puro, containing a gene encoding TurboGFP, driven by the CMV promoter. Co-transfection of this control along with the Lentiviral Packaging Mix provides fast visual confirmation of successful transfection and transduction.
Positive Control: Transduction with shRNA targeting reporter vector	MISSION TurboGFP shRNA Control Transduction Particles, Catalog Number SHC004V The TurboGFP shRNA transduction particles are produced from the sequence-verified lentiviral plasmid, pLKO.1–Puro vector containing shRNA that targets TurboGFP (Catalog # SHC004). These particles can be used as a positive control to quickly visualize knockdown. This TurboGFP shRNA has been experimentally shown to reduce GFP expression by 99.6% in HEK 293T cells after 24 hours. Because this shRNA targets TurboGFP, and it does not target any human or mouse genes, it can also be used as a negative non-target control in shRNA experiments.

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