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Product Information

CRISPR & MISSION® Lentiviral Packaging Mix

Catalog Numbers **SHP001 and SHP002**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
The Lentiviral Packaging Mix is an optimized formulation of two plasmids expressing the key HIV packaging genes and a heterologous viral envelope gene.

Lentiviral particles are generated from three main components:

1. The packaging vector, which contains the minimal set of lentiviral genes required to generate the virion structural proteins and packaging functions.
2. The vesicular stomatitis virus G-protein (pCMV-VSV-G) envelope vector, which provides the heterologous envelope for pseudo-typing and allows these lentiviral particles to efficiently deliver the transfer sequence of interest to a wide variety of cell types, including primary and non-dividing cells.
3. The lentiviral transfer vector, which contains the sequence of interest as well as the *cis* acting sequences necessary for RNA production and packaging.

The Lentiviral Packaging Mix contains the first two components; it is designed to be co-transfected along with a compatible lentiviral transfer vector in order to create high-titer pseudo-typed lentiviral particles used for downstream transduction applications.

In both packaging systems, the pseudo-typing with VSV-G broadens the viral tropism associated with the virus.[1] Therefore, these lentiviral particles can efficiently deliver the transfer sequence of interest to a wide variety of cell types, including primary and non-dividing cells.[2,3]

The packaging system used to produce the particles has many features of a third generation system to enhance biosafety.[4] Some of these features are described in Table 1.

Table 1.
Biosafety Features of the Lentiviral Packaging System

Feature	Result
Multi-plasmid approach	No single plasmid contains all the genes necessary to produce packaged lentivirus. Resultant particles are replication-incompetent
Deletion in U3 portion of 3′ LTR which eliminates the promoter-enhancer region	Avoids promoter interference issues and further negates the possibility of viral replication
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

The Lentiviral Packaging Mix and the lentiviral transfer vectors can be easily co-transfected into a mammalian production cell line. HEK293T cells, ATCC® Number CRL-11268, are recommended as they are readily transfected and have been demonstrated to consistently produce a high titer of virus.[6]

Recombinant lentiviruses produced with the Lentiviral Packaging Mix have not been shown to produce replication competent viral particles because of designed safety features. Users should consult and observe their own institutional guidelines for working with such viral systems.

Reagents
Lentiviral Packaging Mix, 0.25 or 1.7 ml
Catalog Numbers SHP001 and SHP002

Reagents and equipment required, but not provided

- Lentiviral-based transfer vector(s).
- FuGENE®6 Transfection Reagent, Roche Diagnostics or User preferred
- HEK293T producer cells ATCC Number CRL-11268
- Serum-free DME medium
- DME medium supplemented with 10% fetal bovine serum and 4 mM L-glutamine.
- HIV-1 p24 Antigen ELISA Kit, ZeptoMetrix, ZMC Catalog Number 0801111

Follow distributor's instructions for culturing and maintaining the producer cell line. It is recommended to use the lowest passage number of cells as possible for transfection experiments.

Precautions and Disclaimer
For R&D use only. Not for drug, household, or other uses.

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.[7] Follow all published RGL-2 guidelines for handling and waste decontamination. Also, use extra caution when packaging lentiviral particles that express shRNA-targeting genes involved in cell cycle control, e.g., tumor suppressor genes.

Storage/Stability
The Lentiviral Packaging Mix should be stored at –20 °C. Avoid multiple freeze thaw cycles.

Procedure
Overview

Day 1	Day 2	Day 3	Day 4	Day 5
Plate HEK293T cells	Transfect cells with packaging mix and transfer vector	Re-feed cells with fresh medium	First lentiviral particle harvest	Second lentiviral particle harvest; p24 assay

Day 1:
Plate the HEK293T cells in complete DME medium, supplemented with 10% fetal bovine serum and 4 mM L-glutamine, 24 hours prior to transfection. Cells should reach 50-70% confluency the day of transfection. Recommended cell seeding densities for different plate formats are outlined in Table 2.

Table 2.
Recommended Cell Densities for Various Formats

Format	Initial Number of Cells Plated	Final Volume of Viral Harvest (Assumes Two Harvests)
96 well plate	16,000–20,000 cells/well	200 µL
60 mm dish	5×10^5 cells/dish	8.0 mL
100 mm dish	1×10^6 cells/dish	22 mL
6 well plate	2.3×10^5 cells/well	6.0 mL
T75 flask	1.8×10^6 cells/flask	30 mL
T225 flask	5.4×10^6 cells/flask	90 mL

Day 2:
1. Thaw the vial of Lentiviral Packaging Mix at room temperature. Once thawed, place on ice.

Preparing Volume (in µL) of Lentiviral Packaging Mix
To calculate the amount of Lentiviral Packaging Mix needed for the experiment, refer to Table 3. Prepare excess volume of Lentiviral Packaging Mix based on format used, in order to compensate for pipetting error.

2. Within a sterile polypropylene tube(s) add the calculated volume of Lentiviral Packaging Mix for each transfection.
3. Keep tube(s) on ice.

Table 3.
Volume of Lentiviral Packaging Mix Required for Various Formats

Format	Lentiviral Packaging Mix
96 well plate	1 µL/well
60 mm dish	10 µL/dish
100 mm dish	26 µL/dish
6 well plate	4.6 µL/well
T75 flask	34 µL/flask
T225 flask	107 µL/flask

Preparing Amount (in μg) of Transfer Vector
To calculate the amount of transfer vector needed for the experiment, refer to Table 4. Prepare excess amount of transfer vector based on format used, in order to compensate for pipetting error.

4. Add the calculated volume of transfer vector for each transfection to the sterile polypropylene tube(s).
5. Keep tube(s) on ice.

Table 4.
Amount of Transfer Vector Required for Various Formats

Format	Transfer Vector
96 well plate	0.1 μg/well
60 mm dish	1.0 μg/dish
100 mm dish	2.6 μg/dish
6 well plate	0.5 μg/well
T75 flask	3.4 μg/flask
T225 flask	10.7 μg/flask

Preparing Volume of FuGENE 6 Transfection Reagent/Serum-Free Medium Solution

Note: FuGENE 6 transfection reagent has to be prepared in combination with serum-free DME medium. If using another transfection reagent follow manufacturers recommendation.

To calculate the amount of FuGENE 6 transfection reagent/serum-free medium, refer to Table 5. Prepare an excess volume based on format used, in order to compensate for pipetting error.

Table 5.
Volume of DME Serum-free Medium and FuGENE 6 Transfection Reagent Required for Various Formats

Format	DME Serum-free Medium	FuGENE 6 Transfection Reagent
96 well plate	15 μL/well	0.6 μL/well
60 mm dish	70 μL/dish	6 μL/dish
100 mm dish	182 μL/dish	16 μL/dish
6 well plate	30.3 μL/well	2.7 μL/well
T75 flask	237 μL/flask	21 μL/flask
T225 flask	710 μL/flask	62 μL/flask

6. In a sterile polypropylene tube(s), first add the calculated amount of DME Serum-Free medium.
7. To the medium, add the FuGENE 6 transfection reagent.
   Note: Use polypropylene material tube(s) rather than polystyrene because FuGENE 6 transfection reagent tends to stick to polystyrene.
8. Mix components gently.

Making Transfection Cocktail
Combine all transfection cocktail components together in one polypropylene vial.

9. Add volume of Lentiviral Packaging Mix per one sample based on the recommendation in Table 3.
10. Add amount of transfer vector per one sample based on the recommendation in Table 4.
11. Add volume of DME serum-free medium/ FuGENE 6 per one sample based on the recommendation in Table 5.
12. Mix gently by pipetting up and down.
13. Incubate transfection cocktail for 15 minutes at room temperature.
14. Add entire transfection cocktail volume from each polypropylene well(s), plate(s), dish(es), or flask(s) to the corresponding well(s), plate(s), dish(es), or flask(s) containing HEK293T cells.

Day 3
Re-feed with fresh medium

15. Pre-warm enough complete medium to 37 °C to be used for feeding of the cells.
16. At 16 hours post-transfection, remove medium from the transfected cells (avoid disturbing cells) and replace with an equal volume of the pre-warmed complete medium.
17. Add the volume of DME complete medium to each well, dish, or flask based on the recommendations in Table 6.

Table 6.
Volume of DME Complete Medium Required for Various Formats

Format	DME Complete Medium
96 well plate	100 μL/well
60 mm dish	5 mL/dish
100 mm dish	10 mL/dish
6 well plate	2-3 mL/well
T75 flask	15 mL/flask
T225 flask	45 mL/flask

18. Incubate cells in incubator (37 °C and 5% CO_2) for an additional 24 hours.

Day 4

First harvest of Viral Particles

19. Pre-warm enough complete medium to be used for feeding of the cells.
20. Between 36-48 hours post-transfection, collect viral particles by carefully removing the medium and placing it in a collection tube.

    The first harvest of viral particles can be stored at 2-8 °C for 24-48 hours. For long-term storage, freeze at –70 °C.
    Note: Multiple freeze thaw cycles of viral particles may reduce the infectious viral titer by 20-50% per cycle.
    Note: Harvest time points should be optimized depending upon cell health, passage number, and medium composition at the time of transfection.
21. Add an equal volume of fresh pre-warmed complete medium to cells and incubate for an additional 24 hours

Day 5

Second harvest of Viral Particles

22. Between 60-72 hours post-transfection collect viral particles by carefully removing the medium (if desired, pool with first harvest).

    The second harvest of viral particles can be stored at 2-8 °C for 24-48 hours. For long-term storage, freeze at –70 °C.
    Note: Multiple freeze thaw cycles of viral particles may reduce the infectious viral titer by 20-50% per cycle.

23. Titer viral particles by performing the HIV p24 Antigen ELISA assay immediately after pooling (see manufacturer's protocol).

References

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4. Dull, T., *et al*., A third generation lentivirus vector with a conditional packaging system. *J. Virol*., **72**, 8463-8471 (1998).
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8. Whither RNAi? *Nature Cell Biology*, **5**, 489-490 (2003).

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Troubleshooting Guide

Problem	Cause	Solution
Low viral titer or no viral titer determined in HIV p24 Antigen ELISA assay	Lentiviral Packaging Mix was not added to the transfection mix.	Repeat experiment.
	Lentiviral Packaging Mix was not stored properly.	Check if Lentiviral Packaging Mix was stored at –20 °C upon receiving.
	Lentiviral Packaging Mix went through multiple freeze-thaw cycles.	After receiving, generate aliquots of the Lentiviral Packaging Mix and store at –20 °C until ready for use.
	The target gene is essential for cell growth and viability.	Be sure that target gene is not essential for cell growth and viability.
	shRNA construct is larger then 2 kb.	shRNA clones larger then 2 kb can negatively impact production of lentiviral particles.
	Low yield of shRNA plasmid DNA.	The viral-based vectors are known to be difficult to purify. We recommend streaking the bacterial stock on LB/carbenicillin plate to isolate a single colony and DNA purification with GenElute™ HP Midiprep Kit, Catalog Number NA0200, or GenElute HP Maxiprep Kit, Catalog Number NA0300.
	Used poor quality plasmid DNA.	Do not use plasmid DNA from a mini-prep for transfection. Use DNA purification with Sigma's GenElute plasmid purification kits mentioned above.
	Seeding density of cells was not optimal.	Follow cell density recommendations in Table 1.
	Media and reagents were stored improperly.	Store media and reagents at temperatures designated by the manufacturers.

Control Selection Table

Sigma's recommended controls for any shRNA experiment are closely aligned with the controls suggested in the *Nature Cell Biology* editorial.[8]

Recommended Control	Objective
Negative Control: Untreated Cells	Untreated cells will monitor cells culture growth conditions and provide a reference point for comparing all other samples.
Negative Control: Transfection with empty pLKO.1 vector, containing no shRNA insert	MISSION pLKO.1-puro Control Vector, Catalog Number SHC001. This vector does not contain an shRNA insert. Particles generated with this construct serve as a useful negative control during transduction. This controls for transduction effects without subsequent activation of the RNAi pathway.
Negative Control: Transduction with Non-targeting shRNA	MISSION Non-Target shRNA Vector Control, Catalog Number SHC002 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: Transfection with positive reporter vector	MISSION TurboGFP Control Vector, Catalog Number SHC003 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: Transfection with shRNA targeting reporter vector	MISSION TurboGFP shRNA Control Vector, Catalog Number SHC004 The TurboGFP shRNA vector consists of the pLKO.1–Puro vector, containing shRNA that targets TurboGFP, and can be used as a positive control to quickly visualize knockdown. This TurboGFP shRNA Control Vector has been experimentally shown to reduce GFP expression by 99.6% in HEK 293T cells after 24 hours. Because this vector 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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