Imanis Cellective LV Targeting System

Cellective LV Targeting Kit User Guide

Catalog #ICT-LTK
Version 1.0 (Jan 2026)

Product Description

The Imanis Cellective LV Targeting System is a collection of reagents used to generate lentiviral vectors (LV) targeted to specific cellular receptors. The system has three core components:

- **Cellective LV Packaging Mix**: used in combination with LV transfer plasmid for transient transfection of HEK293T to produce reprogrammable LV; compatible with second- and third-generation transfer plasmids.
- **Cellective Targeting Adapter**: used to label reprogrammable LV with specific receptor Targeting Adapter.
- LV transfer plasmid: transfer plasmid encoding the desired LV genome; used for co-transfection with **Cellective LV Packaging Mix**.

Components are available for purchase individually or as a kit (**Cellective LV Targeting Kit**). Available sizing facilitates a broad range of *in vitro*- and *in vivo*-scale applications.

Products and Storage

Product	Cat. No.	Available sizes	Storage[1]
Cellective LV Packaging Mix	ICT-LPM	Small: 10 µg Medium: 50 µg Large: 250 µg	≤ -20°C
Cellective CD3 Targeting Adapter	ICT-TAC3	Small: 14 µg Medium: 70 µg Large: 350 µg	≤ -80°C
Cellective LV Targeting Kit • Cellective LV Packaging Mix • Cellective CD3 Targeting Adapter • pLV-SFFV-eGFP Transfer Vector Control	ICT-LTK	Small Medium Large	See requirements for individual components

Expected Yields[2]

- Small: ~$2x10^{10}$ lentiviral particles (LVP).
- Medium: ~$1x10^{11}$ lentiviral particles (LVP).
- Large: ~$5x10^{11}$ lentiviral particles (LVP).

[1]Shelf-life is 1 year under proper storage conditions.
[2]When packaging pLV-SFFV-eGFP.

Required materials not supplied with the Cellective LV Targeting System

- Temperature & CO_2 controlled incubator
- Phase contrast microscope
- Biosafety Cabinet, Class II A2
- Adjustable micro pipettors
- Laboratory mixer
- Automated cell counter or hemocytometer
- Nucleic acid free tubes
- 10-cm tissue culture dishes
- HEK293T Cells (recommend ATCC CRL-3216)
- Opti-MEM (recommend Gibco # 31985070)
- PEI Pro (1 mg/mL) (recommend Polyplus #101000033 *or equivalent*)
    - Alternative transfection reagents: See FAQ
- Fetal bovine serum
- High glucose DMEM
- Trypan blue (or alternative cell counting dye)
- 50 mL conical tube
- 0.45-micron PES filter

Additionally, a p24 ELISA is required for quantification of packaged lentiviral particles. Commercial ELISA kits are available; Imanis also offer p24 titration services. Contact support@imanislife.com.

Procedural Notes

- Optimal cell health is critical to LV yield. Avoid use of cells not maintained in log-phase growth or sub-cultured for > 15 passages from time of thaw.
- Complete media consists of High Glucose DMEM supplemented with 10% FBS and 1x penicillin/streptomycin.
- The Packaging of Reprogrammable LV protocol is written for use with 10-cm culture dishes. Volumes can be scaled to accommodate other sized culture dishes if required. Expected LV yield is $2x10^{10}$ LVP from three 10-cm culture dishes. The Cellective LV Packaging Mix provides enough material for:
    - 10 µg = 3x 10-cm dishes
    - 50 µg = 15x 10-cm dishes
    - 250 µg = 75x 10-cm dishes
- For optimal labeling of Cellective LV with Targeting Adapter, LV preparations should be titered by p24 ELISA prior to labeling. The Targeting Adapter is provided in excess and is used at a fixed ratio.
- See also **Considerations and Troubleshooting**.

Procedure: Packaging of reprogrammable LV

1. **Preparation of Cells (Day 1)**

1.1 Seed HEK293T cells in 10-cm tissue culture plates in complete media.

	1 plate	*n* plates
Number of Cells	**$3.85x10^6$**	***n* x $3.85x10^6$**
Total Cell Preparation Volume	**11 mL**	***n* x 11 mL**
Seeding Volume/Plate	**10 mL**	***n* x 10 mL**
Volume Lentivirus Recovered	**~5 mL**	**~*n* x 5 mL**

1.2 Incubate at 37°C with 5% CO_2 overnight (*minimum 16 hours, see additional information in step 2.1).*

2. **Transfection (Day 2)**

2.1 Check the seeded cells under a microscope. For optimal LV production, cells should be at ~70% confluency at the time of transfection. *Cell seeding volumes are designed to yield plates at ~70% confluency at 24 hours after seeding. However, the time required to reach 70% confluency can vary depending on cell passage and density at time of seeding.*

2.2 Prepare **Transfection Mix A**. Vortex PEIpro immediately prior to use.

	1 plate	*n* plates
PEIpro (1 mg/mL)	**10 µL**	***n* x 10 µL**
Opti-MEM	**340 µL**	***n* x 340 µL**

2.3 Prepare **Transfection Mix B**.

	1 plate	*n* plates
Cellective LV Packaging Mix	**12 µL**	***n* x 12 µL**
LV transfer plasmid*	**2 µg**	***n* x 2 µg**
Opti-MEM	**To 175 µL total vol.**	***n* x 175 µL total vol.**

*For **pLV-SFFV-eGFP Transfer Vector Control**, use 8 µL/plate of the provided reagent.

2.4 Vortex (or mix by pipetting) **Transfection Mix A** and **B** and combine contents into a single tube. Vortex to mix, then briefly spin down.

2.5 Incubate at ambient temperature for 15 minutes.

2.6 Add transfection mix dropwise to the cell plate. Briefly rock to ensure coverage, then incubate at 37°C with 5% CO_2 for 8-16 hours.

2.7 Examine the cells by microscope. Cells should be attached and growing in an even monolayer between 70 to 90% confluent.

2.8 Remove culture media from the plate by aspiration and replace with 6 mL of pre-warmed Opti-MEM. Return the plate(s) to the incubator.

- If using the **pLV-SFFV-eGFP Transfer Vector Control**, ≥95% of the cells should be GFP-positive at 24 hours after transfection.

3. **LV Harvest (Day 4)**

3.1 Transfer culture supernatant to conical tubes. *Pool supernatants from plates transfected with the same plasmid combinations.*

3.2 Centrifuge at 800 x *g* for 10 minutes at 4°C to pellet cell debris.

3.3 Carefully remove the supernatant and filter it through a 0.45 µm PES filter.

3.4 Aliquot LV and store at ≤ -65˚C. Limit freeze-thaw cycles (*see FAQs)*.

4. **LV Titration**

- LV should be titrated by p24 ELISA. Titer differences between p24 ELISA kits are common. The GenScript #L00938 Lentivirus Titer p24 ELISA Kit yields similar titers to Imanis' p24 titration assay.
- To determine the total number of lentiviral particles (LVP) per milliliter from the concentration of p24 (pg/mL), multiply the p24 (pg/mL) concentration by 10,000.

Procedure: Labeling Cellective LV with Targeting Adapter

1. Thaw LV and **Cellective Targeting Adapter** on ice.
2. Refer to the lot-specific certificate of analysis provided with the **Cellective Targeting Adapter** for the supplied concentration.
3. Combine **Cellective Targeting Adapter** and LV at a ratio of **0.6 µg Targeting Adapter to 1x10^9 LVP** and mix gently by pipetting or inverting the tube.
4. Incubate for 1 hour at 22°C ± 4°C.

- Use labeled LV immediately for experiments or freeze ≤ -65°C.

LV Analysis

Effective labeling of LV particles can be confirmed by Western blot analysis. Recommended sample loading is between 5x10^7 and 2x10^8 LVP per well depending on the gel setup. For detection of the G-fusogen, use antibody Alpha Diagnostic International #VSNG13-S. Labeled G will be around 100 kDa, while unlabeled G will appear around 65 kDa. For efficient targeting, labeled G should be detected but represent less than 20% of the total G present on the LV.

LV specificity can be confirmed by transducing both receptor positive and receptor negative cells with increasing volumes of labeled LV.

Considerations and Troubleshooting

Parameter	Considerations
Optimizing LV yield	• The Cellective LV Packaging Mix is compatible with other transfection reagents and protocols but has been optimized for use with the PEIpro transfection protocol in this guide. • If using a different transfection reagent, refer to the manufacturer's instructions for the recommended ratio of transfection mix to total plasmid. Further optimization may be required. • To use a different transfection protocol, replace the packaging and envelope plasmids with Cellective LV Packaging Mix. Further optimization may be required. • For optimal packing of large transfer vectors (> 10kb), increase the dose of transfer plasmid and transfection reagent by 5%.
Poor LV Yields	• Ensure cells are healthy; aim to use cells between passage 3 and 15 post thaw. • Lower titers are common with larger transgenes. Keep transfer plasmids as small as possible by removing unnecessary sequences. • Certain transgenes, especially transmembrane proteins, can interfere with LV packing. Consider using cell-specific promoters to reduce transgene expression with producer HEK293T cells. • Lower titers may be an artifact of the selected p24 assay, as p24 assays are poorly calibrated between manufacturers. The GenScript #L00938 Lentivirus Titer p24 ELISA Kit yields similar titers to Imanis' p24 titration assay.
LV Concentration	• To concentrate LV for downstream applications, perform concentration prior to labeling with Targeting Adapter. • After filtering the LV supernatant, centrifuge at 12,000 x *g* for 4 hours at 4°C. Carefully remove the supernatant and resuspend the LV pellet in DPBS. • PEG can also be used to concentrate LV, but protocols may require optimization.

Considerations and Troubleshooting cont.

Parameter	Considerations
p24 Titration	• The Cellective Targeting System has been optimized to use a set amount of Targeting Adapter for a given number of lentiviral vector particles (LVP). Too much or too little Targeting Adapter can negatively impact the functional titer of the labeled LV. • It is highly recommended that LV be titered by p24 ELISA prior to labeling with Targeting Adapter. However, if the number of LVP is not known, labeling at a range of different Targeting Adapter levels should be performed and tested using a functional transduction assay. • p24 assays are poorly calibrated between manufacturers. The GenScript #L00938 Lentivirus Titer p24 ELISA Kit yields similar titers to Imanis' p24 titration assay.
LV Stability	• LV should be aliquoted, stored ≤ -65˚C, and thawed at the time of labeling. While freeze-thaw cycles should be limited, stability testing has demonstrated little loss in particle titer with up to three freeze-thaw cycles. The recommended workflow is to freeze LV immediately after production and thaw and label immediately before use in downstream experiments. • Labeled LV can be frozen and thawed for later use; to maintain functional titer, do not exceed a single freeze-thaw cycle. • Products are good for up to 1 year from receipt if properly stored. Components should be aliquoted upon first thaw and should not undergo more than three freeze-thaw cycles.
Poor Specificity	• Perform western blot analysis on labeled particles to confirm the presence of programmed G (programmed G will appear around 100 kDa, while unprogrammed G will appear around 65 kDa). For efficient targeting, reprogrammed G should be detected but represent less than 20% of the total G present on the LV. • Confirm target receptor expression on cells being transduced. • Try titrating the amount of labeled LV being used for transduction to optimize on-target transduction while minimizing off-target transduction.