Lentiviral Vector Production
Streamlined gene delivery systems.

PRODUCTION METHODS

Lentiviral Vector Construction:
1. Clone gene of interest into a modified Lentiviral vector.
2. Purify the constructed Lentiviral vector plasmid and packaging plasmids.

To Produce Lentiviral Particles by Transient Transfection In 10 mL Cell Culture Dish:

1. Day 0: One day prior to the transfection, plate 293T cells in 10 mL DMEM/10% FBS at a density of 2 x 10^6 per 100-mm tissue culture plate. Incubate.

2. Day 1: On the day of transfection, change culture medium with 10 mL fresh medium 1 hour prior to the transfection.

3. Mix DNAs used for Lentiviral particle production in a sterile 6 mL polypropylene tube.
   I. Adjust the volume to 437 µl with TE79/10.
   II. Add 63 µl of 2 M CaCl_2 and mix well.
   III. Add 500 µl of 2 x HBS with constant agitation.
   IV. Sit the mixture at room temperature for 30 minutes to allow calcium phosphate-DNA to precipitate.

4. Add the precipitate by drop into tissue culture plates in which 293T cells are at least 80–90% confluent.
   Tips to titer: The cell density is critical for vector production. The best results are obtained when the plate is 90% confluent on the day of transfection. Vi-Cell for precise cell counting can help you reach the ideal cell confluence to increase titer.

5. After 6–8 hours, replace the culture medium with 6 mL fresh DMEM/10% FBS and continue the incubation.

6. Day 2–4: Collect the culture supernatant and replace by 6 mL fresh culture medium. Filter the collection through a sterile 0.4 µm syringe filter, and store at ultra-low temperature (ULT).

SUPPORTING PRODUCTS

OPTIMA XPN-100 with NVT rotors (100, 90, 65 & 65.5)

MICROFUGE 16, GeXP

OPTIMA MAX-XP with MLA rotors (150 & 130) and TLA rotors (120.1 & 120.2)

AVANTI J-26S XP with JA rotors (10, 14) and JLA rotors (16.250, 10.500), JA rotors (17, 20 & 25.5)

ALLEGRA X-15R, ALLEGRA X-14, ALLEGRA X-30

Vi-CELL
Polyethylene Glycol (PEG) Purifies and Concentrates Lentiviral Particles:

1. Mix thawed collection with 40% PEG solution to a final PEG concentration of 10%. Incubate the mixture in ice for 3–6 hours.
2. Spin at 2,000 x g for 30 minutes.
3. Discard the supernatant, disperse viral particle pellet by gentle pipetting in 1/20 of the original harvest volume of PBS (Phosphate Buffered Saline) or media of your choice.
4. Place the tubes into buckets. Weigh and balance them.
5. Spin at 100,000 x g (24,500 RPM) at 4°C in a SW 32 Ti rotor for 90 minutes, in a Beckman Optima X Series ultracentrifuge.
6. Remove the supernatant by inversion of the tubes or pipetting; be careful not to dislodge the viral pellet.
7. Re-suspend the pellet in PBS or the media of your choice.
8. Pipette up and down or shake for a few minutes, if necessary, to fully dissolve the pellet.
9. Aliquot and store at desired temperature; ultra-low temperature (ULT) storage is recommended for long term.

**IMPROVED PROCESS:**

<table>
<thead>
<tr>
<th>Rotors</th>
<th>Tube</th>
<th>Part Number</th>
<th>Adapter</th>
<th>Process Advantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>SW 55 Ti</td>
<td>3.2 mL g-Max, konical and BioSafety with Quick-Seal</td>
<td>358647</td>
<td>355535 and 358153</td>
<td>Increased concentration, biosafety, reduced sample volume</td>
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<tr>
<td>SW 32.1 Ti</td>
<td>4.5 mL g-Max and BioSafety with Quick-Seal</td>
<td>356562</td>
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<td>Reduced sample volume, biosafety</td>
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<td>8.0 mL g-Max and BioSafety with Quick-Seal</td>
<td>344621</td>
<td>355579</td>
<td>Reduced sample volume, biosafety</td>
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<tr>
<td>SW 32 Ti</td>
<td>15 mL g-Max and BioSafety with Quick-Seal</td>
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<td>3555356</td>
<td>Reduced sample volume, biosafety</td>
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<td>8.4 mL g-Max, konical and BioSafety with Quick-Seal</td>
<td>358652</td>
<td>3555356 and 358156</td>
<td>Reduced sample volume, biosafety, increased concentration</td>
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<tr>
<td>TLS-55*</td>
<td>2.2 mL Ultra-Clear</td>
<td>347356</td>
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<td>Miniaturization</td>
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<tr>
<td>MLS-50*</td>
<td>5.0 mL Ultra-Clear</td>
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<td>Miniaturization</td>
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<td>SW 41 Ti</td>
<td>13.2 mL Ultra-Clear</td>
<td>344059</td>
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<td>Reduced sample volume</td>
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</tbody>
</table>

*TLS and MLS rotors are used with the Optima MAX-XP tabletop ultracentrifuge.

References: