

# 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  $2 \times 10^6$  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  $\mu$ L with TE79/10.
  - II. Add 63  $\mu$ L of 2 M  $\text{CaCl}_2$  and mix well.
  - III. Add 500  $\mu$ L of 2  $\times$  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  $\mu$ m 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 × 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 × 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:

Rotors	Tube	Part Number	Adapter	Process Advantages
SW 55 Ti	3.2 mL <i>g</i> -Max, konical and BioSafety with Quick-Seal	358647	355535 and 358153	Increased concentration, biosafety, reduced sample volume
SW 32.1 Ti	4.5 mL <i>g</i> -Max and BioSafety with Quick-Seal	356562	355579	Reduced sample volume, biosafety
	8.0 mL <i>g</i> -Max and BioSafety with Quick-Seal	344621	355579	Reduced sample volume, biosafety
SW 32 Ti	15 mL <i>g</i> -Max and BioSafety with Quick-Seal	343664	355536	Reduced sample volume, biosafety
	8.4 mL <i>g</i> -Max, konical and BioSafety with Quick-Seal	358652	355536 and 358156	Reduced sample volume, biosafety, increased concentration
TLS-55*	2.2 mL Ultra-Clear	347356	—	Miniaturization
MLS-50*	5.0 mL Ultra-Clear	344057	—	Miniaturization
SW 41 Ti	13.2 mL Ultra-Clear	344059	—	Reduced sample volume

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

## References:

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3. Steven R. Bartz and Marie A. Vodicka; Production of High-Titer Human Immunodeficiency Virus Type 1 Pseudotyped with Vesicular Stomatitis Virus Glycoprotein—METHODS: A Companion to Methods in Enzymology. 12: pp. 337-342.
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