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 2×10^6 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).

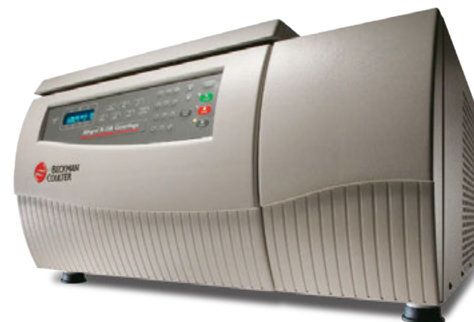
SUPPORTING PRODUCTS

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:

Rotors	Tube	Part Number	Adapter	Process Advantages
SW 55 Ti	3.2 mL *g*–Max, konical and BioSafety with Quick-Seal	358647	355535 and 358153	Increased concentration, biosafety, reduced sample volume
SW 32.1 Ti	4.5 mL *g*–Max and BioSafety with Quick-Seal	356562	355579	Reduced sample volume, biosafety
	8.0 mL *g*–Max and BioSafety with Quick-Seal	344621	355579	Reduced sample volume, biosafety
SW 32 Ti	15 mL *g*–Max and BioSafety with Quick-Seal	343664	355536	Reduced sample volume, biosafety
	8.4 mL *g*–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:

1. Hsin-Lung Lo and Jiing-Kuan Yee; Production of Pseudotype-Retroviral Vectors—Current Protocols in Human Genetics. John Wiley & Sons, Inc. pp. 12.7.1-11: 2007.
2. Jane C. Burns, Theodore Friedmann, Wolfgang Driever, Michelle Burrascano, and Jiing-Kuan Yee; Vesicular stomatitis virus G glycoprotein pseudotyped retroviral vectors: Concentration to very high titer and efficient gene transfer into mammalian and nonmammalian cells—Proc. Natl. Acad. Sci. Vol. 90: pp. 8033-8037.
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.
4. Jiing-Kuan Yee, Atsushi Miyanohara, Patricia Laporte, Kathy Bouic, Jane C. Burns, and Theodore Friedmann; A general method for the generation of high-titer, pantropic retroviral vectors: Highly efficient infection of primary hepatocytes—Proc. Nati. Acad. Sci. Vol. 91: pp. 9564-9568.
5. Richard A. Klinghoffer, Brian Roberts, James Annis, Jason Frazier, Patrick Lewis, Peter S. Linsley, and Michele A. Cleary; An Optimized Lentivirus-Mediated RNAi Screen Reveals Kinase Modulators of Kinesin-5 Inhibitor Sensitivity—ASSAY and Drug Development Technologies. Volume 6: pp. 105-119.
6. Jean-Michel Garcia, Anhui Gao, Pei-Lan He, Joyce Choi, Wei Tang, Roberto Bruzzone, Olivier Schwartz, Hugo Naya, Fa-Jun Nan, Jia Li, Ralf Altmeyer, and Jian-Ping Zuo; High-throughput screening using pseudotyped lentiviral particles: A strategy for the identification of HIV-1 inhibitors in a cell-based assay—Antiviral Research. 81: pp. 239–247.
7. H-L Lo, T Chang, P Yam, PM Marcovecchio, S Li, JA Zaia, and J-K Yee; Inhibition of HIV-1 replication with designed miRNAs expressed from RNA polymerase II promoters—Gene Therapy. 14: pp. 1503–1512.

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