Table of Contents

Safe Handling of Lentivirus

Users of this protocol should fully understand the potential hazards associated with the use of recombinant lentivirus, as well as the precautions and techniques necessary for the safe handling, storage, and laboratory use of infectious lentivirus. For users requiring more information on lentiviruses, retroviruses, and mammalian cell culture, we recommend the following general references:

- *Retroviruses*, ed. by J. M. Coffin, S. H. Hughes & H. E. Varmus (1997, Cold Spring Harbor Laboratory Press, NY). Available on the web at http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=rv.TOC.
- *Culture of Animal Cells, 5th Edition*, by R. I. Freshney (2005, Wiley-Liss, NY).

For more information on Biosafety Level 2 agents and practices, download the following reference:

- *Biosafety in Microbiological and Biomedical Laboratories (BMBL), Fifth Edition*, (February 2007) HHS Pub. No. (CDC) 93-8395. U.S. Department of Health and Human Services Centers for Disease Control and Prevention and NIH. Available on the web at http://www.cdc.gov/biosafety/publications/bmbl5/index.htm.

Purifying Lentivirus

IMPORTANT: *Read this entire protocol before beginning.* Perform all the steps of this protocol using sterile technique, inside a *Biosafety Level 2* tissue culture hood that has been approved for use with lentiviruses.

1. Place a ring stand or column support inside the tissue culture hood to hold the Lenti-X Maxi Column.
2. Remove the top cap from the column. Decant and discard the 20% ethanol storage buffer.
3. Remove the bottom cap from the column and secure the column in the stand or rack. Position a reservoir beneath the column (e.g. a sterile 50 ml conical tube).
4. Pipet 20 ml Lenti-X Equilibration Buffer into the column, making sure to wash the inside of the column to remove residual ethanol. Discard the flowthrough.

   NOTES:
   - While flowing, tap the column gently to dislodge any air trapped beneath the frit at the bottom of the column. The column should run at a rate of 2–3 ml/min, running slightly faster with higher load volumes and more slowly as the buffer volume decreases.
   - Harvest the lentivirus-containing supernatants. CAUTION: Supernatants contain live lentivirus. Pool similar stocks if desired. Centrifuge briefly (500 x g for 10 min) or filter through a 0.45 µm filter. If filtering, use only cellulose acetate or polyenersulfone (PES) (low protein binding) filters. *Do not use nitrocellulose filters.* Nitrocellulose binds surface proteins on the lentiviral envelope and destroys the virus.

5. Add 10X Lenti-X Dilution Buffer to your lentiviral supernatant at a 1:10 ratio (i.e., add 1 ml of buffer to 9 ml of supernatant). Up to 30 ml of diluted supernatant can be applied to the column.

   NOTE: Be sure to save an aliquot of the undiluted supernatant for titration by qRT-PCR, lentiviral p24, or cell-based infection.

6. Load the diluted supernatant onto the column. Collect and save the flowthrough for later titration, if necessary. Store at –80°C.

7. Wash the column with 10–20 ml of Lenti-X Wash Buffer. Collect and save the flowthrough for later titration, if necessary. Store at –80°C.
8. Add 3 ml of Lenti-X Elution Buffer to the column. Collect and save the eluate. This fraction will contain the majority of your lentivirus.

   NOTE: The lentiviral sample is eluted in high salt buffer and may require desalting or buffer exchange prior to use. We recommend using a 10 ml PD-10 Column (GE Healthcare).

9. Titrate the virus immediately and/or store at –80°C.

Contact Us	
Customer Service/Ordering	**Technical Support**
tel: 800.662.2566 (toll-free)	tel: 800.662.2566 (toll-free)
fax: 800.424.1350 (toll-free)	fax: 650.424.1064
web: www.clontech.com	web: www.clontech.com
e-mail: orders@clontech.com	e-mail: tech@clontech.com

Notice to Purchaser

This document has been reviewed and approved by the Clontech Quality Assurance Department.