I. General Considerations

A. Storage

Store at 4°C upon receipt. DO NOT FREEZE.

B. Stability

Stable for 1 year from date of receipt under proper storage conditions.

C. Required Materials

- Lenti-X Accelerator (Cat. Nos. 631256 or 631257)
- Magnetic Separator for Cell Culture (available separately as Cat. No. 631255, or combined with Lenti-X Accelerator in the Lenti-X Accelerator Starter Kit, Cat. No. 631254)
- Complete medium without antibiotics

D. Cell Plating & Transduction Conditions

- Plate your cells one day prior to transduction. They should be 60–80% confluent on the day of transduction. Cultures that are less than 50% confluent at the time of transduction may lose viability.

  IMPORTANT: Antibiotic-free medium (no Penicillin-Streptomycin) is recommended during transduction to minimize any decrease in viability. Please do not use any Penicillin G and Streptomycin (Pen-Strep) in the media during transduction. Pen-Strep may safely be added to the media after at least two passages, when the density of beads is typically lowered.

- The recommended amount of Lenti-X Accelerator (magnetic bead suspension) is typically sufficient to bind the number of viral particles produced using standard lentivirus production protocols. (1×10^5 to 1×10^8 IFU/ml). Table 1 provides the recommended range of bead and virus suspension volumes and cell densities for different sizes of culture vessel (plates and dishes).

NOTE: We recommend testing a few different bead volumes with a fixed virus dosage to achieve the highest transduction efficiency possible with your particular cell type.

Table I. Recommended Plating & Transduction Conditions for Different Sizes of Culture Vessel

Culture Vessel	Growth Area (cm^2)	Volume of Medium (ml)	Cell Density	Volume of Beads (µl)	Volume of Virus (µl)
96-well plate	0.32	0.2	$0.04–0.1 \times 10^5$	0.5–2	5–20
24-well plate	1.88	0.5	$0.2–0.6 \times 10^5$	2–8	20–80
12-well plate	3.83	1	$0.4–1.2 \times 10^5$	4–16	40–160
6-well plate	9.4	3	$1–3 \times 10^5$	8–30	80–300
60-mm dish	21	5	$2–6 \times 10^5$	16–64	160–640
100-mm dish	55	10	$5–15 \times 10^5$	32–128	320–1280

II. Procedure

Perform all of the following steps under sterile conditions:

1. Mix Lenti-X Accelerator magnetic bead suspension by either pipetting up and down several times or by vortexing to achieve a uniform bead suspension.
2. Add the recommended volume (Table 1) to a sterile 1.5-ml microfuge tube.
3. Add the corresponding volume of virus-containing supernatant (Table 1) to the same tube and mix by gently pipetting up and down. Your virus may be resuspended in PBS or complete media.
4. Incubate the bead-virus mixture at room temperature or on ice for 20–30 min. During this incubation, tap the tube gently every 5 min to mix.

   NOTE: The volume of magnetic bead suspension relative to that of virus suspension should be 10% or higher (see Table 1). A lower ratio requires a longer binding time.

5. Add the magnetic particles-virus mixture dropwise to the cells to be transduced. Rock the culture plate/dish gently to distribute the particles evenly across all the cells.
6. Place the culture plate/dish on top of the magnetic plate (i.e., Magnetic Separator for Cell Culture) for 5 min at room temperature or in a 37°C CO_2 incubator.
7. Change the media and then remove the culture from the magnetic plate. Cultivate cells under standard conditions. Any residual beads will be lost as the culture is passaged.

Contact Us	
Customer Service/Ordering	**Technical Support**
Telephone: 800.662.2566 (toll-free)	Telephone: 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

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