Lentivirus Packaging Protocol

Table 1: Reagent Requirements for Lentivirus Production

Vessel	Seeding Density	DNA Mix			Transfection Mix	
		Transfer Plasmid	Packaging Mix	Serum-Free DMEM	Transfection Reagent	Serum-Free DMEM
15 cm	12.5 x 10^6	30 µg	60 µl	2.5 ml	160 µl	2.5 ml
10 cm	5.0 x 10^6	10 µg	20 µl	1 ml	80 µl	1 ml
6-well	1.0 x 10^6	2.5 µg	5 µl	100 µl	16 µl	100 µl

*Note: the specified amounts and protocol apply to **abm** products and may differ when using alternative products.

Day 1

1. Seed an appropriate number of HEK293T cells (Cat. No. LV010) into a desired vessel according to Table 1 using complete growth media, and incubate at 37°C with 5% CO_2 overnight.

Day 2

1. Verify that cells have reached 70-80% confluence before proceeding with transfection.
2. Based on your selected vessel, use Table 1 to prepare two solutions, the DNA Mix and Transfection Mix in 1.5 ml tubes. The DNA Mix includes: Transfer Plasmid, Packaging Mix (Cat. No. LV003) and Serum-Free DMEM (Cat. No. TM003). The Transfection Mix includes: Transfection Reagent (Cat. No. G2500) and Serum-Free DMEM. Incubate at room temperature for 5 min.
3. Prepare the Transfection Complex by combining the DNA Mix and Transfection Mix together, and then incubate at room temperature for 20 min.
4. Add Serum-Free DMEM to the Transfection Complex (800 µl for 6-well, 4 ml for 10 cm dish, 5 ml for 15 cm dish).

5. Aspirate media from the vessel and gently add the Transfection Complex to the cells. Incubate at 37°C with 5% CO_2 for 5-8 h.
6. Add complete growth media to the vessel (1 ml for 6-well, 4 ml for 10 cm dish, 5 ml for 15 cm dish) and incubate at 37°C with 5% CO_2 overnight.

Day 3

1. Aspirate media from the vessel and add an appropriate amount of complete growth media. Incubate at 37°C with 5% CO_2 for 24 h.

Day 4

1. Collect the supernatant from the vessel into a centrifuge tube and centrifuge at 1500 x g for 15 min at 4°C. Transfer the clarified supernatant to a fresh tube.
2. Apply the clarified supernatant over a PES 0.45 µm sterile filter. Use lentivirus immediately or store at 4°C (short term) or -80°C (long term).
3. Optional: A second harvest can be performed by adding an appropriate amount of complete growth media to the remaining cells from Day 4 Step 1 and incubating at 37°C with 5% CO_2 for an additional 24 h. The following day, perform Day 4 Step 1-2 procedure and combine the filtered supernatant with the first harvest.
4. Recommended: Add ViralEntry™ Transduction Enhancer (Cat. No. G515) to the culture media when using the lentivirus to enhance downstream transduction efficiency.