



3rd Generation Packaging Mix

Cat. No. LV053

Store at -20°C.

Product Description

abm's **Third Generation Packaging Mix** offers a high-safety, high-efficiency solution for lentivirus production. This system requires four plasmids for viral particle production: 1) Transfer Plasmid containing your gene of interest, 2) Packaging Plasmid containing structural proteins Gag and Pol, 3) Packaging Plasmid containing structural protein Rev, and 4) Envelope Plasmid containing transmembrane glycoprotein VSV-G. The ready-to-use 3rd Generation Packaging Mix combines optimized Packaging and Envelope Plasmids for high viral titers, superior biosafety and reliable gene delivery performance.

Packaging Mix Component	Quantity
pLenti-P3A	200 µl, 500 ng/µl
pLenti-P3B	
pLenti-P3C	

Additional Materials Required (not included)

Material	Recommended Product	Cat. No.
HEK293T Cell Line	293T Cells	LV010
Serum-Free DMEM	PriGrow III Medium	TM003
Transfection Reagent	DNAfectin™ Plus	G2500
Transduction Enhancer	ViralEntry™ Transduction Enhancer	G515

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 the recommended products and may differ when using alternative products.

Day 1

- Seed an appropriate number of HEK293T cells into a desired vessel according to Table 1 using complete growth media, and incubate at 37°C with 5% CO₂ overnight.

Day 2

- Verify that cells have reached 70-80% confluence before proceeding with transfection.
- 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 and Serum-Free DMEM. The Transfection Mix includes: Transfection Reagent and Serum-Free DMEM. Incubate at room temperature for 5 min.
- Prepare the **Transfection Complex** by combining the DNA Mix and Transfection Mix together, and then incubate at room temperature for 20 min.
- 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).
- Aspirate media from the vessel and gently add the Transfection Complex to the cells. Incubate at 37°C with 5% CO₂ for 5-8 h.
- 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% CO₂ overnight.

Day 3

- Aspirate media from the vessel and add an appropriate amount of complete growth media. Incubate at 37°C with 5% CO₂ for 24 h.

Day 4

- 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.
- 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).
- 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% CO₂ for an additional 24 h. The following day, perform Day 4 Step 1-2 procedure and combine the filtered supernatant with the first harvest.
- Recommended: Add **ViralEntry™ Transduction Enhancer** to the culture media when using the lentivirus to enhance downstream transduction efficiency.