# 2nd Generation Packaging Mix

## Cat. No. LV003
Store at -20°C.

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

| Packaging Mix Component | Quantity |
|---|---|
| pLenti-P2A | 200 µl, 500 ng/µl |
| pLenti-P2B | |

## 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**
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% $CO_2$ 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 and Serum-Free DMEM. The Transfection Mix includes: Transfection Reagent 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** to the culture media when using the lentivirus to enhance downstream transduction efficiency.

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