

Retrovirus Packaging Protocol

Recommended Protocol (Cat.No. E-510)

Version 1.0
2026

*This procedure is for packaging Retrovirus using HEK293T cells, **Retrovirus Packaging Combo Mix** (Cat. No. E-510), and **DNAfectin™ Plus Transfection Reagent** (Cat.No. G2500). Crude viral supernatant is expected to yield 10-20 ml of 10^6 IU/ml, and additional concentration by ultra-centrifugation is recommended if higher titers are required.*

Transfection

- 1.1 **Day 0:** 18 to 24 hours prior to transfection, seed low passage HEK293T cells at a density of 5×10^5 cells per 10 cm plate in 10 ml of DMEM + 5% FBS + 1% P/S. Incubate the cells at 37°C in a 5% CO₂ incubator until cells are 70% confluent at the time of transfection.

Note: Using low passage number cells are important in the success of viral packaging. In addition, do not allow HEK293T cells to overgrow - always subculture the cells at 80% confluency. If the cells overgrow, their morphology will change and transfection efficiency may be reduced.

- 1.2 **Day 1:** Verify that cells have reached 70-80% confluence before proceeding with transfection. Follow **Table 1** to prepare two solutions in separate 15 ml tubes:

- **Solution A (DNA Mix)** includes: Transfer Plasmid, Packaging Mix and Serum-Free DMEM.
- **Solution B (Transfection Mix)** includes: Transfection Reagent and Serum-Free DMEM.

Table 1. Reagent volumes for the two solutions that make up the **Transfection Complex Mixture**:

Solution A (DNA mix)	Volume
Retroviral Vector DNA (0.5 µg/µl)	30 µl (15 µg)
Retrovirus Packaging Combo Mix (Cat.No. E-510)	36 µl
Serum-free DMEM	2.5 ml

Solution B (Transfection mix)	Volume
DNAfectin Plus Transfection Reagent (Cat.No. G2500)	40 µl
Serum-free DMEM	2.5 ml

- 1.3 Mix each solution well **individually**, then incubate solutions **separately** at room temperature for 5 minutes.
- 1.4 Combine **Solution A** and **Solution B** in the same tube, gently pipette up and down to ensure homogeneity. Incubate the **Transfection Complex Mixture** at room temperature for 20 minutes.
- 1.5 Slowly add the **Transfection Complex Mixture** in one corner of the 10 cm dish, then gently distribute around the plate. Incubate cells at 37°C in 5% CO₂ for 5-8 hours.
- 1.6 After 5-8 hours, to one corner of the dish, gently add 5 ml fresh growth media (DMEM + 10% FBS). Incubate the cells at 37°C in 5% CO₂.
- 1.7 **Day 2:** Change media, and continue incubating the cells at 37°C in 5% CO₂.

Harvest

*Typically viral supernatant is harvested on **Day 4**, however an additional optional harvest can be done a day earlier on **Day 3** to increase the amount of virus collected. This is especially useful for harvesting a larger volume of viral supernatant for ultra-centrifugation.*

- 2.1 **Day 3:** (Optional, skip to Day 4 if only doing a single harvest): Collect the viral supernatant from the vessel into a centrifuge tube and centrifuge at 1500 x g for 15 minutes at 4°C. To the culture vessel, add 10 mL of complete growth media to the culture vessel and continue incubating at 37°C with 5% CO₂, until Day 4.
- 2.2 After centrifugation, transfer the clarified viral supernatant to a new tube and store at 4°C, and execute the filtration step (Step 2.4) for both days' harvest together.
- 2.3 **Day 4:** Collect the viral supernatant from the vessel into a centrifuge tube and centrifuge at 1500 x g for 15 minutes at 4°C. Transfer the clarified supernatant to a new tube (or the tube collected from Day 3).
- 2.4 Filter the clarified viral supernatant with a PES 0.45 µm filter into a sterile 50 ml tube.
- 2.5 Aliquot crude retroviral supernatant to appropriate size volumes to use immediately or store at -80°C.