Retrovirus Packaging Kit

Cat. #: P905

Application: Kit for packaging retrovirus. This product is for research use only.

Product Size: 10 reactions, 100mm dish per reaction

Product Description: This kit provides a rapid and convenient method for producing high titers of replication-incompetent retroviruses in only 48 hours.

This Moloney Murine Leukemia Virus (MMLV)-based packaging system and VSV-G possesses the ideal host range required for your target cells.

This kit contains high efficient transfection reagent and a ready-to-use retroviral packaging mix.

Separate expression of the essential viral structural genes ensures safety and control over the recombinant constructs by minimizing the risks that arise during cell division and reducing the chance of producing replication-competent virus.

- Rapid virus production
- Ready-to-use
- Wide range target cells
- Low risk of replication-competent

Shipping / Storage: Ship at **4°C,** store at **4°C and -20°C** (see below for instruction)

Shelf Life: 6 months

Component:

Component	Cat. #	Amount	Storage
293T Transfection Reagent	P903	450 µl	4°C
Retrovirus Packaging Mix	P905P	200 µl	–20°C, avoid freeze-thaw cycles

Remark: Follow the recommended NIH BSL-2 guidelines for all materials containing Retrovirus.

Materials Needed: (not provided) Tissue culture plate 10 cm, 293T (or 293FT) cells to transfect, pRetro expression vector (contains your gene of interest), Serum-free DMEM

Protocol (example of producing virus in 100mm petri dish)

Day 1: Plating cells

1. 24 hours before transfection, coat 100mm dishes with **6 mL 1x Gelatin** for 60 min.
2. Aspirate gelatin, and plate **~5 X 10^6** fast growing 293T cells/plate in evenly distribution manor in 10 ml medium.

Note: Before plating, pipet the 293T cell suspension **vigorously** to achieve single cells.

Day 2: Transfection

1. Check to make sure the cells are 70-80% confluent.
2. **Change medium: 2 hours before** transfection: remove culture medium and add **10 ml fresh** complete culture medium (antibiotic does not influence the result).
3. In 1.5 ml tube 1 add:
   7.5 µg Retrovirus vector (contains your gene of interest)
   15 µg Retrovirus packaging mix
   500 µl DMEM (serum-free, High Glucose)

   Pipet up and down to mix well
4. In 1.5 ml tube 2 Add:
   45 µl 293T Transfection Reagent
   500 µl DMEM (serum-free, High Glucose)

   Gently pipet up and down to mix well
5. Incubate at room temperature (20–25°C) for 1 minute.
6. Add tube 2 into tube 1, pipet up and down several times. **Vortex** for **10 seconds**.
7. Incubate for **15 minutes** at **room temperature** (20–25°C).
8. Add the incubated mixture **drop-wise** to the cells, and **gently rock / swirl** the plate.
9. Return the cells to 37°C incubator with 5% CO_2.

Day 3: Add Retrovirus Titer-Up to enhance the virus titer

(Optional) Add **20 µl Retrovirus Titer-Up** (500x, Cat. #: P909) to the culture medium.
Return the plates to the cell culture incubator, and incubate at for another 24 hours.

Day 4: Collect virus (The virus can be harvested twice at 48 hours and 72 hours post transfection.)

1. Collect virus supernatant in a 50 ml sterile, capped conical centrifuge tube. Centrifuge it at **3,000rpm** for **15 minutes** at 4°C to remove cell debris. Filter the clear supernatant through **0.45 µm** sterile filter.
2. The filtered clear supernatant is virus soup. The virus is ready for infection, purification, or it can be stored as a viral stock at -80°C for your future applications (for **up to 3 months**). Aliquot volumes are preferred for storage to reduce the viral titer loss from freeze-thaw cycles.
3. If second harvest is needed, add 10 ml of complete medium to the cells after the first harvest. (Optional) Add **20 µl Retrovirus Titer-Up** (500x, Cat. #: P909) to the culture medium. Put the dish back to a 37°C incubator.
4. The 2nd harvest can be done on Day 5, following steps 1-3 above.
5. (Optional) Concentrate virus: add **5X Retrovirus Concentration Solution** (101Bio, Cat. #: P905C) to the viral supernatant (volume of Retrovirus Concentration Solution vs. volume of viral supernatant = 1:4) and mix thoroughly. Put the mixture to 4°C refrigerator overnight and spin the virus pellet down next day. Please refer our P905C user manual for details.

– The end –