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| Name: | Retroviral Packaging Mix |
| Cat. #: | P905P |
| Application: | For packaging retrovirus. This product is for research use only. |
| Usage: | For 10 cm dish of retrovirus packaging, mix 7.5 µg of retroviral expression vector with 15 µl of retroviral packaging mix |
| Product Description: | <p>The Retroviral Packaging Mix provides a rapid and convenient method for producing high titers of replication-incompetent retroviruses. This Moloney Murine Leukemia Virus (MMLV)-based packaging system possesses the ideal host range required for your target cells. It is a ready-to-use retroviral packaging mix, which contains VSVG and produces high titer retroviruses in only 48 hours. Separate expression of these 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 viruses.</p> <p>This Mix is an optimized formulation for producing high-titer pantropic retrovirus.</p> <ul style="list-style-type: none">• Achieve average titer of 10⁷ infectious units/ml with transient transfection.• Simple cotransfection of the retroviral packaging mix and the expression vector containing your gene of interest.• Produce self-inactive and pantropic retrovirus. |
| Shipping / Storage: | Ship at 4°C or RT ; Store at -20 °C . This product is stable for 6 months when stored at -20 °C . Freeze-thaw cycles should be minimized by dividing into single-use aliquots. Store aliquots in the -20 °C freezer until ready for use. |
| Shelf Life: | 6 months |
| Volume: | 200 µl |
| Remark: | Follow the recommended NIH BSL-2 guidelines for all materials containing Lentivirus. |

Protocol

| <u>Condition</u> | <u>Amount</u> |
|---|-----------------------------------|
| Tissue culture plate size | 10 cm (one per lentiviral vector) |
| Number of 293T cells to transfect | 4 × 10 ⁶ cells |
| Amount of retroviral packaging mix | 15 µl |
| Amount of pRetro expression vector | 7.5 µg |
| Amount of Transfection reagent (Cat.# P903) | 45 µl |
| Amount of serum free DMEM | 500 µl |

DAY 1: **1.** Seed 293T cells in 10cm dishes (~4 X 10⁶ cells per 10cm dish).

DAY 2: **2.** Check to make sure the cells are 70-80% confluent.

3. For each 10cm dish prepare transfection complex as follows:

a) 1.5ml Tube A: Dilute 7.5 µg DNA plasmid and 15 µl retroviral packaging mix in 0.5 ml serum-free medium (e.g.

DMEM). Mix by pipetting.

b) 1.5ml Tube B: Dilute 45 µL of transfection reagent (101BioCat.# P903) in 0.5 ml serum-free medium and mix gently.

c) Add Transfection reagent/DMEM into DNA/DMEM solution. Mix by vortexing for 5-10 seconds then incubate the DMEM-plasmid-Transfection mixture at room temperature for 15 minutes.

4. Add the complete transfection complex from step 3 drop-wise to the cells, and swirl the dish to disperse the transfection complex evenly in the plate.

5. Incubate the plate at 37°C overnight.

DAY 3: **6.** Replace the supernatant with 10 ml fresh media. Note: You may supplement the culture medium with 20 µl of Retrovirus 10X Titer-Up Reagent (101Bio, Cat.# P909) to enhance the virus titer.

7. Incubate at 37°C for 24 hours.

DAY 4 (1st Harvest): **8.** Collect the supernatant that contains retroviruses into a 50ml sterile, capped conical centrifuge tube.

9. Centrifuge supernatant at 3000 rpm for 15 minutes at 4°C to pellet cell debris.

10. Filter the clear supernatant through a low-protein binding 0.45-µm sterile filter.

11. The virus is ready for infection, purification, or it can be stored as a viral stock at -80°C for your future applications. Aliquot volumes are preferred for storage to reduce the viral titer loss from freeze-thaw cycles.

12. If second harvest is needed, add 10 ml of complete medium to the cells after the first harvest and put the dish back to 37°C incubator. Note: You may supplement the culture medium with 20 µl of Retrovirus 10X Titer-Up Reagent (101Bio, Cat.# P909) to enhance the virus titer.

DAY 5 (2nd Harvest)

13. The second harvest can be done on Day 5, following steps 8-11.

14. To concentrate virus, add a 1 volume Retrovirus Concentration Solution (101Bio, Cat.# P904C) to 4 volumes of the viral supernatant, and mix thoroughly.

15. Put the mixture to 4°C refrigerator overnight and pellet the viruses next day. Refer the user manual for details.

Related products: (on Product Page, see Virus Products)

Virus Transduction Enhancer

- [PV100 Virus Transduction Enhancer](#)
- [PV500 Virus Transduction Enhancer](#)

Lentivirus

- [Lentivirus 10X Titer-Up](#)
- [293T Transfection Reagent for Lentivirus Packaging](#)
- [Lentivirus Packaging Kit](#)

Retrovirus

- [Retrovirus 10X Titer-Up](#)
- [293T Transfection Reagent for Retrovirus Packaging](#)
- [Retrovirus Packaging Kit](#)