Product Specification Sheet

Product Name	**Retrovirus Packaging Mix**
Description	The Retrovirus Packaging Mix provides a rapid and convenient method for producing high titers of replication-incompetent retroviruses, which possess the ideal host range required for your target cells. This Moloney Murine Leukemia Virus (MMLV)-based packaging system is a ready-to-use retrovirus packaging mix that 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, reducing the chance of producing replication-competent viruses.
Catalog Number	VP200
Size	200 µl
Shipping	Ambient Temperature
Storage and Stability	Store at -20° C. This product is stable for 6 months when stored as directed. Freeze-thaw cycles should be minimized by dividing into single-use aliquots. Store aliquots in the -20° C freezer until ready for use.
Usage	For 10 cm dish of retrovirus packaging, mix 7.5 µg of retroviral expression vector with 15 µl of retrovirus packaging mix.
Quality Control	Each lot of retrovirus packaging mix is functionally tested in transfection assays using human embryonic kidney 293 cells.
Safety Consideration	Follow the recommended NIH guidelines for all materials containing BSL-2 organisms. Always wear gloves, use filtered tips and work under a biosafety hood.
Restricted Use	For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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Protocol (VP200)

Materials Needed

Tissue culture plate	10 cm (one per retroviral vector)
293FT cells to transfect	4 x 10^6 cells
Rentivirus Packaging Mix	15 µl
pRetro expression vector	7.5 µg
NanoFect™ (Cat. # NF100)	45 µl
Serum-free DMEM	500 µl

Procedure

Day 1

1. Seed 293T cells in 10 cm dishes (add ~4 x 10^6 cells per dish).

Day 2

2. Check to make sure the cells are 70-80% confluent.
3. For each 10 cm dish prepare transfection complex as follows:
   a. 1.5 ml Tube A: Dilute 7.5 µg DNA plasmid and 15 µl Retrovirus Packaging Mix in 0.5 ml serum-free medium (e.g., DMEM). Mix by pipetting.
   b. 1.5 ml Tube B: Dilute 45 µl of NanoFect™ in 0.5 ml serum-free medium and mix gently.
   c. Add NanoFect™/DMEM into DNA/DMEM solution. Mix by vortexing for 5-10 sec and then incubate the DMEM-plasmid-NanoFect™ mixture at room temperature for 15 min.
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 cells at 37° C overnight.

Day 3

6. Replace supernatant with 10 ml fresh media.
   Note: You may supplement the culture medium with 20 µl of ViralBoost™ (Cat. # VB100) 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 50 ml sterile, capped conical centrifuge tube.
9. Centrifuge supernatant at 3000 rpm for 15 min 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.

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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 a 37° C incubator.
    Note: You may supplement the culture medium with 20 µl of ViralBoost™ (Cat. # VB100) 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 Precipitation Solution (Cat. # VC200) to 4 volumes of the viral supernatant, and mix thoroughly.
15. Put the mixture to a 4° C refrigerator overnight and pellet the viruses the next day. Please refer to the user manual for details.

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