Product Name: Retrovirus **10X Titer-Up Reagent**

Cat. #: P909, P909S (test sample), Cat.# at Fisher Sci: *NC1692641*

Application: Increase Retrovirus titer up to **10 times** in virus packaging procedure. This product is for research use only.

Product Size: P909: 1 mL (for packaging 500 mL retrovirus soup); P909S: 0.1 mL

Product Description: This product is a novel recipe of small molecules designed for effective virus packaging.

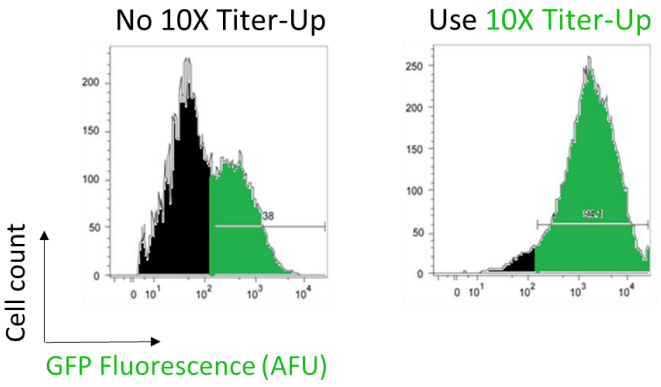
- ✓ Increase virus **titer** by **10 times**
- ✓ Increase **viral RNA** transcription
- ✓ Increase virus particle **packaging efficiency**

Shipping / Storage: Ship at room temperature and store in **4°C**

Shelf Life: 12 months

Remark: Each lot of 10X Titer-Up reagent is **functionally tested** in virus production experiment using 293T cells.

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



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 **~5.5 X 10^6** fast growing 293T cells per plate, in 10 ml medium.

Note: Before plating, pipet the 293T cell suspension vigorously to achieve single cells.
Plate the cells in evenly distribution manor.

Day 2: Transfection (using 101Bio "293T Trasfection Reagent", Cat. #: P903)

1. **Change medium: 2 hours before** transfection, remove culture medium and add **8 mL fresh** complete culture medium (with 10% serum / without antibiotics)
2. In tube 1 add: ~20 µg DNA (virus vector and packaging mix [101Bio, Cat. #: 905C])
   500 µL DMEM (serum-free, High Glucose)

   Pipet up and down to mix well
3. In tube 2 Add: 45 µL 293T Transfection Reagent for Retrovirus Packaging
   500 µL DMEM (serum-free, High Glucose)

   Gently pipet up and down to mix well
4. Incubate at room temperature (**20 ~ 25°C**) for 3 min.
5. Add tube 2 into tube 1, pipet up and down several times. **Vortex** for **10 seconds**.
6. Incubate for **15 minutes** at **room temperature**.
7. Add the incubated mixture **drop-wise** to the cells, and **gently rock / swirl** the plate.
8. Return the cells to 37°C incubator with 5% CO_2.

Day 3: Add 10X Titer-Up

Add **20 µl** of **10X Titer-Up** (500x) to the medium. Return the plates to the cell culture incubator.

Day 4: Collect virus

1. Collect virus supernatant twice at **48 and 72 hours** post transfection into a 50mL sterile FALCON tube. Centrifuge at **3,000rpm** for **15 minutes** at 4°C to remove cell debris. Filter the clear supernatant through **0.45 µm** syringe tip filter.

2. The filtered clear supernatant is virus soup. Use it immediately or aliquot into sterile 1.5-mL tubes and store at **-80°C**, for **up to 3 months.**

Note: No side effect 10X Titer-Up reagent on gene expression has been detected when directly transduce 293T cells. It may be various on different cell types (cell lines). A pilot test on the side effect of 10X Titer-Up is advised on sensitive cells.

3. (Optional) To concentrate virus, add 1 volume of 5X Lentivirus/Retrovirus Concentration Solution (101Bio, Cat. #: P904C, Cat.# at FisherSci.com is NC3242189) to 4 volume of the viral supernatant (volume of Lenti-Retrovirus Concentration Solution vs. volume of viral supernatant = 1:4) and mix thoroughly. Put the mixture in the 4C refrigerator overnight and spin the virus pellet down the next day. Please refer to our P904C user manual for details.

Note: No side effect 10X Titer-Up reagent on gene expression has been detected when directly transduce 293T cells. It may be various on different cell types (cell lines). A pilot test on the side effect of 10X Titer-Up is advised on sensitive cells.

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