293T Transfection Kit for **Retrovirus Packaging**

Cat. #: P903

Storage: 4°C

Shelf life: 6 months

Product Size: **1 mL** 293T Transfection Reagent for Retrovirus Packaging

Product Description (This product is for research use only)

This transfection reagent is a **unique recipe** for delivery of **Retrovirus packaging DNA** to 293T cells at highest efficiency, to produce high titer retrovirus.

- ✓ Higher efficiency than lipid-based transfection Reagents
- ✓ **No media changes** needed
- ✓ **Serum compatible**
- ✓ **One-step** incubation - 15 minutes

Reagent required not provided in this kit: serum-free DMEM with High Glucose

Table 1. Suggested Reaction Matrix

Culture Dish	Area (cm^2)	Cell Number	Medium (mL)	DNA (µg)	Transfection Reagent (µL)	DMEM (µL)
6 - Well	10	9.5×10^5	1.0	4	7.55	200
60 mm	25	2.5×10^6	3.0	7-12	15-24	300
100 mm	55	5.5×10^6	8.0	15-25	35-45	500
150 mm	150	15×10^6	20	45-75	60-80	1000

Protocol (example of producing virus by transfecting 293T cells in **one 100mm petri dish**. Refer to Table 1 for other plates or dishes)

Notice: Transfect cells at 70-90% confluency for high transfection efficiency and low toxicity. Before plating, pipet the 293T cell suspension vigorously to achieve single cells. Plate the cells in evenly distribution manor.

Day 1: Plating cells

1. **Plating: 18 to 24 hours** prior to transfection, coat 100mm dishes with **6 mL 1x Gelatin** (101Bio, Cat. #: P910) for 60 min. Aspirate gelatin, and plate **5.5×10^6** fast growing 293T cells.

Day 2: Transfection

2. **Change medium: 2 hours before** transfection, remove culture medium and add **8 mL fresh** complete culture medium (with 10% serum / without antibiotics)

3. In tube 1 add:

7.5	µg	Retrovirus vector DNA	(Containing your gene of interest)
15	µg	Retrovirus Packaging Mix	(101Bio, Cat. # P905P)
500	µL	DMEM	(serum-free, High Glucose)

Pipet up and down to mix well

4. 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

5. Incubate at room temperature (**20–25°C**) for **3 min**.

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**.

8. Add the incubated mixture **drop-wise** to the cells, and **gently swirl** the plate to disperse evenly in the plate.

9. Return the cells to 37ºC incubator with 5% CO_2.

Day 3: Add Titer-Up to increase virus titer

Add **20 µl** of Retrovirus **Titer-Up** (101Bio, Cat. #: 909) to the medium. Return the plates to the cell culture incubator.

Day 4: Collect virus

10. 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 **4ºC** to remove cell debris. Filter the clear supernatant through **0.45 µm** syringe tip filter.

11. 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.**

12. (Optional) To 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 –

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