Cat # SL100489-RAW Store at 4 °C

GenJet™ In Vitro DNA Transfection Reagent for RAW 264.7 Cell

----- A General Protocol for Transfecting plasmid DNA to RAW 264.7 Cell

- ☐ 100 µl
- ☐ 500 µl
- ☐ 1000 µl

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This product is for laboratory research ONLY and not for diagnostic use

Introduction:

GenJet™ In Vitro DNA Tranfection Reagent (Ver. II) is upgraded version of GenJet™ In Vitro DNA Tranfection Reagent. With a new chemistry, more DNA condensing groups were released in the new version compared with old version GenJet™, leading to 3~20 times more efficient in DNA delivery. GenJet™ (Ver. II) for Raw 264.7 Cell was formulated and pre-optimized specifically for transfecting RAW 264.7 cells.

Important Guidelines for Transfection:

- Maintain the same seeding conditions between experiments. Use low-passage cells and make sure that cells are healthy and greater than 90% viable before transfection.
- For maximum transfection efficiency, we recommend using GenJet™ Transfection Buffer (1x) to dilute plasmid DNA and GenJet™ Reagent.

Standard Transfection of Raw 264.7 Cells

Step I. Preparation of Working Solution of GenJet™ Transfection Buffer:

GenJet™ Transfection Buffer (5x) is provided as 5x concentrated stock solution. To make working solution, dilute one part of the stock solution with 4 parts of ddH_2O into a sterile bottle. The working solution is table at 4 °C~RT for 12 months.

Step II. Transfection of RAW 264.7 Cells:

Use this procedure to transfect plasmid DNA into RAW 264.7 cells in a 24-well format. For other formats, see **Scaling Up or Down Transfections** below. All amounts and volumes are given on a per well basis.

- The day of transfection, count the cells to determine culture density. Plate 1x10^5 cells per well in 0.5 ml of complete growth medium. Cell density should be ~70% confluent on the day of transfection.

  Note: GenJet™ reagent is NOT interfered by serum and antibiotics, therefore serum and antibiotic containing medium can be used during the entire experiment.
- For each well of cells to be transfected, dilute 0.5 µg plasmid DNA with 50 µl working solution of GenJet™ transfection buffer prepared from **Step I**. Pipette up and down to mix.
- Add 1.5 µl of GenJet™ Reagent directly to the diluted plasmid DNA solution followed by mix gently and incubate for ~15 minutes at RT.

  Note: Never keep the complex longer than 30 minutes.
- Add the 50 µl transfection mix to the cells drop wise. Gently rock the plate back and forth and return the plate to CO2 incubator.
- Replace transfection medium by cell growth medium ~5 hours after transfection when necessary.
- Gene silencing is usually measured 24~48 hours post transfection.

Storage: GenJet™ Transfection Reagent is stable for up to 12 months at 4 °C. This item shipped at ambient temperature

Scaling Up or Down Transfections

To transfect RAW 264.7 cells in different tissue culture formats, refer to the table below (Given on a per well basis).

Culture Vessel	Growth Medium (ml)	Cells per Well	Transfection Buffer (µL)	Plasmid DNA (µg)	GenJet™ Reagent (µL)
96-well	100	2 x 10^4	10	0.1	0.3
24-well	500	1 x 10^5	50	0.5	1.5
12-well	1.0	2 x 10^5	75	1.0	3.0
6-well	2.0	5 x 10^5	100	2.0	6.0
60 mm	4.0	8 x 10^5	300	5.0	15
10 cm/T-75 lask	8.0	2 x 10^6	800	9.0	27