Cat # SL100489-WEHI Store at 4 °C

GenJet™ In Vitro DNA Transfection Reagent for WEHI-231 Cell

----- A General Protocol for Transfecting plasmid DNA to WEHI-231 Cell

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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 WEHI-231 CeII was formulated and pre-optimized specifically for transfecting WEHI-231 ceII.

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 WEHI-231 Cell

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 ddH2O into a sterile bottle. The working solution is stable at 4 °C-RT for 12 months.

Step II. Transfection of WEHI-231 Cells:

Use this procedure to transfect plasmid DNA into WEHI-231 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 ~80% 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 <u>Step I</u>. Pipette up and down to mix.

 Add 1.5 µI 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 μ I 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 WEHI-231 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 |