Cat # SL100489-RIN Store at 4 °C

GenJet™ In Vitro DNA Transfection Reagent for Rin Related Cells (Ver. II)

----- A Protocol for Transfecting Rin and Rin Related Cells

☐ 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 3~4 times more efficient in DNA delivery. GenJet™ (Ver. II) for transfection of Rin family cells was pre-optimized for tanasfecting Rin and related cells.

Procedures for Transfecting Rin Family Cells:

Step I. Prepare Low Serum Transfection Medium:

For optimal transfection efficiency, transfection must be performed in low level serum transfection medium which is DMEM with High Glucose supplemented with final 1% fetal bovine serum (FBS) without antibiotics. Prepare transfection medium by adding FBS to DMEM with High Glucose to make final 1% FBS freshly.

Step II. Cell Seeding:

Cells should be plated 18 to 36 hours prior to transfection so that the monolayer cell density reaches to the optimal ~90% confluency at the time of transfection. Remove the complete culture medium and freshly add transfection medium from **Step I** to each well ~60 minutes before transfection.

Important: High cell confluency ~90% on the day of transfection is essential for high efficiency and low toxicity.

Table 1. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Transfection Medium Volume (ml)	Plasmid DNA (µg)	Diluent Volume (mL)	GenJet™ Reagent (µL)
96-well	0.2	0.2	2 x 0.01	0.6
48-well	0.3	0.5	2 x 0.02	1.5
24-well	0.5	1.0	2 x 0.05	3
6-well	1.2	2	2 x 0.1	6
35 mm dish	1.5	2	2 x 0.1	6
60 mm dish	3	5	2 x 0.25	15
10 cm dish	6	7 - 8	2 x 0.5	21 - 24
T75 flask	10	18 - 36	2 x 0.75	54 - 108
250 ml flask	20	50 - 100	2 x 1.25	150 - 300

Step III. Preparation of GenJet™-DNA Complex and Transfection Procedures

For Rin and Rin related cells, the optimal ratio of GenJet™ (µL):DNA (µg) is 3:1. We recommend the GenJet™ (µL):DNA (µg) ratio of 3:1 as a starting point which usually gives satisfactory transfection efficiency with invisible cytotoxicity.

To ensure the optimal size of complex particles, we recommend using serum-free DMEM with High Glucose to dilute DNA and GenJet™ Reagent.

Important: The diluent must be serum-free. Never use OPTI Medium from Invitrogen!

The following protocol is given for transfection in 24-well plates, refer to **Table 1** for transfection in other culture formats. The optimal transfection conditions for Rin cells are given in the standard protocol described below.

- For each well, add 0.5 ml of complete medium with serum and antibiotics freshly ~60 minutes before transfection.
- For each well, dilute 1 µg of DNA into 50 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly to bring drops to bottom of the tube .
- For each well, dilute 3 µl of GenJet™ reagent (Ver. II) into 50 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.
- Add the diluted GenJet™ Reagent immediately to the diluted DNA solution all at once. **(Important: do not mix the solutions in the reverse order !)**
- Vortex- mix the solution immediately and spin down briefly to bring drops to bottom of the tube followed by incubation of 15~20 minutes at room temperature to allow GenJet™-DNA complexes to form.
  Important: Never keep the transfection complex longer than 20 minutes at RT
- Add the 100 µl GenJet™/ DNA complex drop-wise onto the medium in each well and homogenize the mixture by gently swirling the plate.
- Remove GenJet™/DNA complex-containing medium and replace with complete culture medium which contains 10% FBS with antibiotics 18~24 hours post transfection.
- Check transfection efficiency 24 to 48 hours post transfection.

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