Cat # SL100489-L929 Store at 4 °C

GenJet™ In Vitro DNA Transfection Reagent for L929 Cell (Ver. II)

----- An General Protocol for Transfecting L929 Cell

- ☐ 100 µl
- ☐ 500 µl
- ☐ 1000 µl

SignaGen® Laboratories
10075 Tyler Place, Suite 19
Ijamsville, MD 21754
FAX. 301-560-4919
TEL. 301-330-5966
Toll Free. 1-(866)-918-6812
Email: info@signagen.com
Web: www.signagen.com

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 L929 was pre-optimized and conditioned for transfecting L929 cell.

Procedures for Transfecting L929 Cell:

Step I. Cell Culture Before Transfection

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal ~70% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 30~60 minutes before transfection.

Note: High serum levels (10%) with antibiotics usually do not have inhibitory effect on transfection efficiency. We recommend using complete serum/antibiotics-containing medium as a starting point. For maximal efficiency and lower cytotoxicity, perform transfection on cells with high density. We recommend transfecting on cells with ~90% confluency.

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

For different cell types, the optimal ratio of GenJet™ (µL):DNA (µg) is 3:1. To ensure the optimal size of GenJet™/DNA complex particles, we recommend using serum-free DMEM with High Glucose to dilute DNA and GenJet™ Reagent.

Note: Never use serum containing medium (such as Opti MEM) to dilute DNA and GenJet™ reagent. The diluent must be serum-free.

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 L929 cell line as well as a general starting point for optimization are given in the standard protocol described below.

- For each well, add 0.5 ml of complete medium with serum and antibiotics freshly 30~60 minutes before transfection.
- For each well, dilute 0.5 µg of DNA into 25 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly to bring drops to the bottom of the tube.
- For each well, dilute 1.5 µl of GenJet™ reagent into 25 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly to bring drops to the bottom of the tube.
  Note: Never use OPTI-MEM to dilute DNA and GenJet reagent as it may disrupt transfection complex.
- Add the diluted GenJet™ reagent **immediately** to the diluted DNA solution all at once. (**Important: do not mix the solutions in the reverse order !**)
- Immediately pipette up and down 3~4 times or vortex briefly to mix.
- Incubate for ~15 minutes at room temperature to allow GenJet™/DNA complexes to form.
  Note: Never keep the GenJet™/DNA complex longer than 30 minutes.
- Add the 50 µl GenJet™/ DNA mixture 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 fresh complete serum/antibiotics containing medium ~5 hours post transfection.
- Check transfection efficiency 24 to 48 hours post transfection.

Table 1. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Volume (ml)	Plasmid DNA (µg)	Diluent Volume (mL)	GenJet™ Reagent (µL)
48 well plate	0.3	0.25	2 x 0.015	0.75
12 well plate	0.75	0.75	2 x 0.038	2.25
6-well plate	1.0	1	2 x 0.05	3.0
35 mm dish	1.0	1	2 x 0.05	3.0
60 mm dish	2.8	2.5	2 x 0.10	7.5
10 cm dish	5.0	5	2 x 0.25	15
T75 flask	8.0	9 - 12	2 x 0.40	27 - 36
250 ml flask	18	25 - 40	2 x 0.8	75 - 120

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