

GenJet™ In Vitro DNA Transfection Kit for SMC (Ver. II)

----- A Protocol for Transfecting Smooth Muscle 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 Transfection Reagent (Ver. II) is upgraded version of GenJet™ In Vitro DNA Transfection 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 DAN delivery. In combination of a proprietary transfection toxicity removal cocktail, GenJet™ In Vitro Transfection Kit (Ver. II) for smooth muscle cells (SMC) is pre-optimized and pre-conditioned for maximally transfecting primary or immortalized smooth muscle cells without visible cell death.

Procedures for Transfecting SMC:

Step 1. Cell Seeding (see Table 1):

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal ~80% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well ~60 minutes before transfection.

Table 1. A Guideline for Seeding Adherent Cells Prior to Transfection in Different Culture Formats

Culture Dishes	Surface Area (cm ²)	Number of Cells to Seed
T75 Flask	75	3.0 - 6.0 × 10 ⁶
100 mm Dish	58	2.2 - 4.4 × 10 ⁶
60 mm Dish	21	0.9 - 1.8 × 10 ⁶
35 mm Dish	9.6	3.5 - 7.0 × 10 ⁵
6-well Plate	9.6	4.0 - 8.0 × 10 ⁵
12-well Plate	3.5	1.5 - 3.0 × 10 ⁵
24-well Plate	1.9	0.8 - 1.6 × 10 ⁵
48-well Plate	1.0	4.0 - 8.0 × 10 ⁴
96-well Plate	0.3	1.2 - 2.4 × 10 ⁴

Step 2. Preparation of GenJet™-DNA Complex and Transfection Procedures

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

The following protocol is given for transfection in 24-well plates, refer to Table 2 for transfection in other culture formats. The optimal transfection conditions for SMC are given in the standard protocol described below.

- 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.
 - Add the diluted GenJet™ Reagent immediately to the diluted DNA solution all at once.
- (Important: do not mix the solutions in the reverse order !)

Table 2. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Transfection Volume (ml)	Plasmid DNA (µg)	Diluent Volume (mL)	GenJet™ Reagent (µL)
96-well	0.2	0.2	2 × 0.01	0.6
48-well	0.3	0.5	2 × 0.02	1
24-well	0.5	1.0	2 × 0.05	3
6-well	1.2	2	2 × 0.1	6
35 mm dish	1.5	2	2 × 0.1	6
60 mm dish	3	5	2 × 0.25	15
10 cm dish	6	7 - 8	2 × 0.5	21 - 24
T75 flask	10	18 - 36	2 × 0.75	54 - 108
250 ml flask	20	50 - 100	2 × 1.25	150 - 300

- Vortex- mix the solution immediately and spin down briefly to bring drops to bottom of the tube followed by incubation of 15 minutes at room temperature to allow GenJet™-DNA complexes to form. **Note:** Never keep GenJet™-DNA complexes longer than 20 minutes.
- 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 DNA/GenJet™ complex-containing medium next day post transfection followed by addition of complete serum/antibiotics containing medium.
- Check transfection efficiency 24 to 48 hours post transfection.