TransIntro® PEI Transfection Reagent（GMP Grade）

Please read the datasheet carefully prior to use.

Cat. No. FT401

Version No. Version 2.0

Storage: at 2°C-8°C for one year.

Description

TransIntro® PEI Transfection Reagent is a high-charged cationic polymer modified based on 40 KDa linear polyethylenimine (PEI). It can form PEI/DNA complexes with negatively charged DNA . The complexes can adhere to negatively charged cell surface residues, and then enters the eukaryotic cell through endocytosis. This product is manufactured and managed in accordance with GMP standards. Animal derived component free, low toxicity, high transfection efficiency, and simple operation. It is suitable for DNA transfection and multi-plasmid co-transfection of HEK-293 and other cell lines. It can be used in the large-scale recombinant protein expression and virus production.

Features

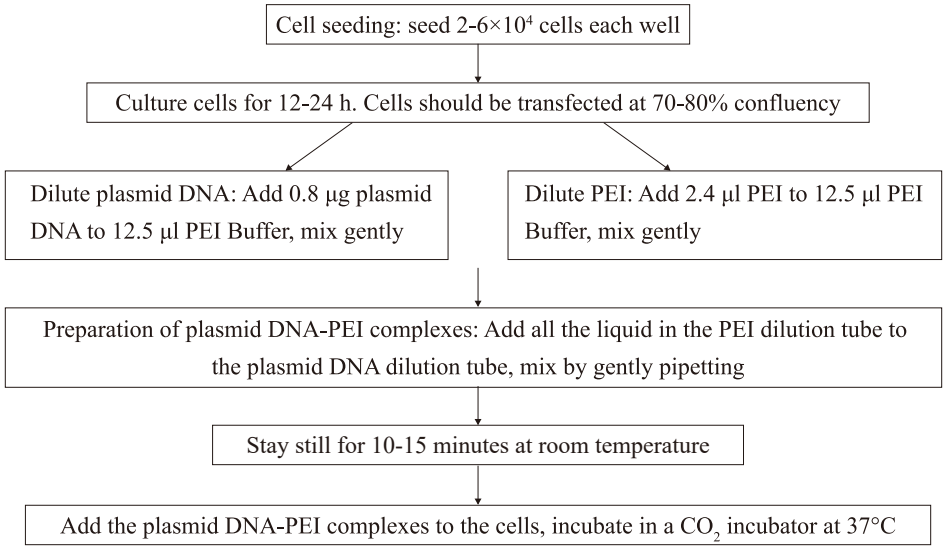
- Wide application: It is suitable for transient transfection and stable transfection of various adherent or suspension cells such as HEK-293.
- High transfection efficiency: It is suitable for high-efficiency transfection of plasmid DNA. Transfection efficiency of HEK-293 is more than 90%.
- Low cytotoxicity: Adherent Cells has a good morphology after transfection. The cell activity rate of suspended cells is more than 90% after 24 hours of transfection.
- Simple operation: No need to change the medium before and after transfection, tolerance with antibiotics and serum.
- GMP Grade: Manufactured and managed in accordance with GMP standards.

Kit Contents

Component	FT401-01	FT401-02
Linear Polyethylenimine (PEI)	1 ml	10 ml
Linear Polyethylenimine Buffer (PEI Buffer)	20 ml	200 ml

Transfection of plasmid DNA

Transfection of adherent cells: Taking transfection of HEK-293 cells in a 24-well plate as an example, the steps are as follows:



Transfection of suspension cells: Taking transfection of 100 ml HEK-293 suspension cells as an example, the steps are as follows:

1. Cell preparation before transfection:

(1) HEK-293 cells are resuscitated and cultured in a 37°C CO_2 constant temperature shaker, and passaged for more than 3 times to restore the cell state;

(2) Seed HEK-293 cells at a density of 3-5×10^5 cells/ml. Continue the culture. When the cells are in the logarithmic growth phase (generally 2-3×10^6 cells/ml) and the cell viability is greater than 98% , perform transfection.

2. Cell Transfection

2.1 The recommended dosage of each component of the transfection system:

a. The dosage of PEI Buffer is 1/20 of the cell culture volume.

b. DNA dosage (μg) = cell density (cells/ml) ÷ 10^6 × culture volume (ml) × 0.45

c. PEI dosage: PEI (μl): DNA (μg) = 3:1

Taking the culture medium volume as 100 ml and the cell density as 2.0×10^6 cells/ml as an example, the calculation is as follows:

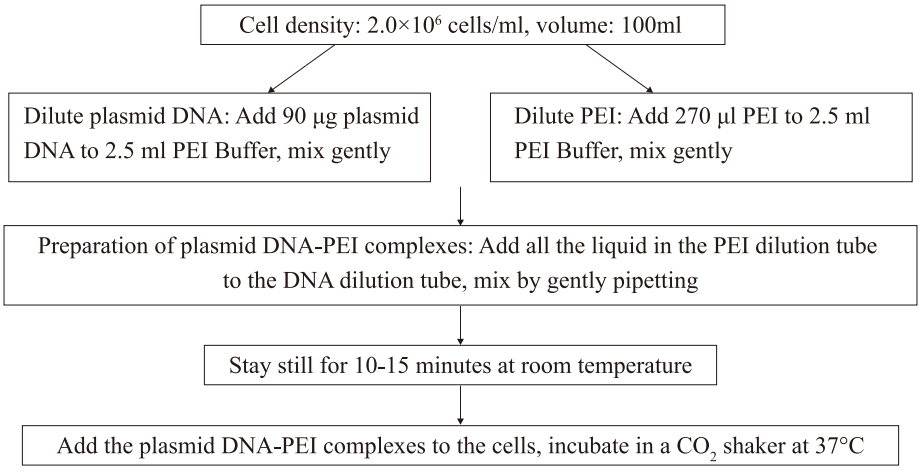
Dosage of PEI Buffer: 100/20=5 ml

DNA dosage: 2.0×10^6÷10^6×100×0.45=90 μg

PEI dosage: 90×3=270 μl

2.2 Transfection steps:

Seed HEK-293 cells at a density of 5×10^5 cells/ml. Perform transfection when the cell density is about 2-3×10^6 cells/ml and the cell viability rate is greater than 98% after culturing it for 2 days . The steps are as follows:



Follow-up experiments

(1) If fluorescent plasmid DNA is transfected, fluorescence should be detected with flow cytometry or fluorescence microscopy 24-48 hours after transfection.

(2) For suspension cells, if the overexpression plasmid DNA is transiently transfected, an appropriate amount of feed medium can be added to enhance the protein expression level about 24 hours after transfection.

(3) If plasmid DNA is stably transfected, an appropriate amount of drugs can be added for drug screening 24-48 hours after transfection.

Causes of poor cell state or low transfection efficiency after transfection

(1) Whether the cells are in good state at the time of transfection (The doubling time of the three generations before transfection is stable. Suspension cells should be have a regular shape and low clumping rate, a cell viability rate greater than 98%, and a cell density of 2-3×10^6 cells/ml. Adherent cells should be have a normal shape and a confluence of about 70%-80% for transfection).

(2) Whether the cell density count of suspended cells is accurate and whether the confluence of adherent cells is determined appropriately.
(3) Whether the plasmid DNA used for transfection is qualified (concentration greater than 200 μg/ml, the ration of A260/280 should be between 1.8-2.0, endotoxin less than 1 EU/μg).
(4) Whether the amount of each component added for transfection is calculated accurately and added correctly.
(5) If the above matters are confirmed correct, suspension cells transfection can consider optimizing the ratio of plasmid DNA and PEI during transfection and the cell density before transfection. It is recommended that the optimized ratio of plasmid DNA and PEI is 1:2-1:5. The cell density before transfection is 2-3×10^6 cells/ml. For adherent cells transfection, you can consider reducing the amount of plasmid DNA and corresponding PEI, or reducing the ratio of plasmid DNA and PEI (the recommended optimization ratio range is 1:2-1:5).

Notes

- PEI reagent is a transparent solution. If there are precipitated crystals before use, please heat it in a water bath at 60-80°C to dissolve it before use.
- The concentration of plasmid DNA used for transfection should be greater than 200 μg/ml, the ratio of A260/280 should be between 1.8-2.0, and endotoxin less than 1 EU/μg.
- After the PEI-DNA complexes is formed, do not pipet or shake vigorously.
- PEI-DNA complexes should be slowly added to the cells, shaking gently while adding.

The amount of medium, plasmid DNA and PEI when transfecting adherent cells in different cell culture plates (for reference only)

Format	Surferace Areaper Well（cm^2）	Volume of Plating Medium（ml）	DNA（μg）	PEI（μl）	PEI Buffer（μl）
96-well	0.3	0.1	0.2	0.3-0.8	5
48-well	1	0.25	0.4	0.6-1.6	13
24-well	2	0.5	0.8	1.2-3.2	25
12-well	4	1.0	1.6	2.4-6.4	50
6-well	10	2.0	4.0	6-16	100
35 mm	10	2.0	4.0	6-16	100
60 mm	20	5.0	8.0	12-32	250
10 cm	60	10	24	24-72	500
T 25	25	6.0	10	10-30	300
T 75	75	20	30	30-90	1000

The suggested amount of PEI when transfecting suspension cells of different densities (take transfection of 100 ml HEK-293 suspension cells in a 500 ml shake flask as an example, for reference only)

Cell Density（10^6 cells/ml）	DNA（μg）	PEI（μl）	PEI Buffer（ml）
2.0	90	270	5
2.2	99	297	5
2.4	108	324	5
2.6	117	351	5
2.8	126	378	5
3.0	135	405	5

For research use only, not for clinical diagnosis.

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