TransIntro® PEI Transfection Reagent（GMP Grade）

Please read the datasheet carefully prior to use.

Cat. No. FT401

Version No. Version 2.1

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, HEK-293F, CHO-S, CHO-K1 and other cell lines. It can be used in the large-scale recombinant protein expression and virus production.

Features

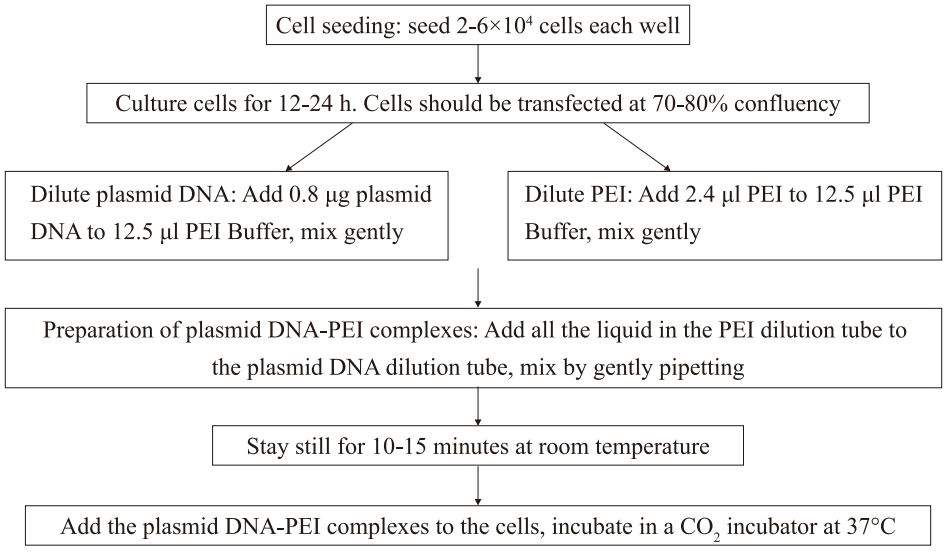
- Wide application: It is suitable for transient transfection and stable transfection of various adherent or suspension cells such as HEK-293, HEK-293F, CHO-S, and CHO-K1.
- High transfection efficiency: It is suitable for high-efficiency transfection of plasmid DNA. Transfection efficiency is greater than 90% in HEK-293 and HEK-293F, and greater than 80% in CHO-S and CHO-K1.
- 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. No freezing required, con venient to use.
- 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 and CHO-S suspension cells as an example, the steps are as follows:

1. Cell preparation before transfection:

(1) HEK-293 or CHO-S cells are resuscitated and cultured in a 37°C CO_2 constant temperature shaker, and passaged for more than 3 times to ensure optimal viability and consistent doubling time;

(2) Seed the cells at a density of 0.3-0.5×10^6 cells/ml. Transfection can be performed when the cells are in the logarithmic growth phase (for HEK-293 cells, 2-5×10^6 cells/ml; for CHO cells, 3-6×10^6 cells/ml) and the cell viability exceeds 98%.

(3) One day before transfection, seed HEK-293 cells at a density of 1-1.5×10^6 cells/ml or CHO-S cells at 2-3×10^6 cells/ml. At the time of transfection, adjust the cell density to the specified density using fresh culture medium.

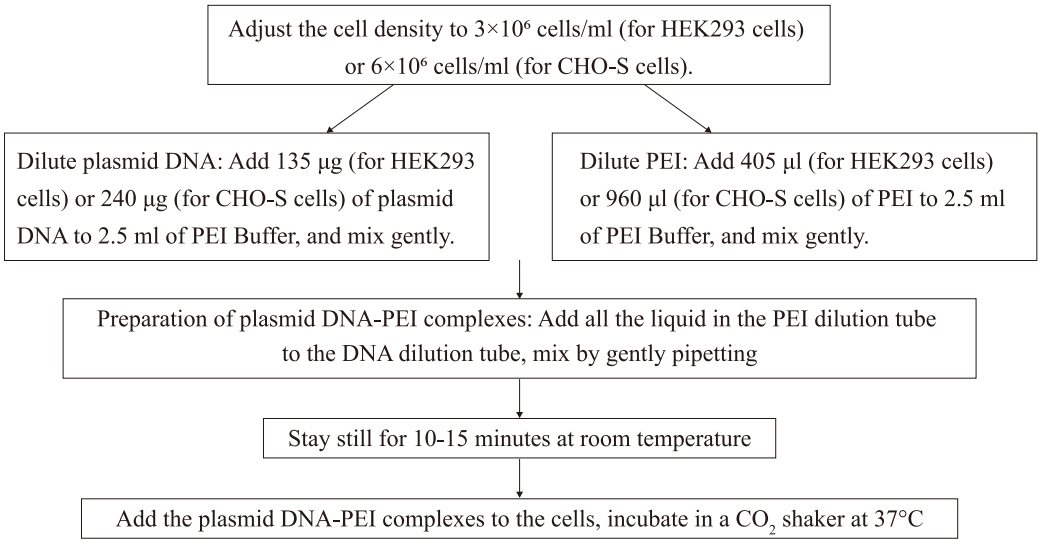
2. Cell transfection

(1) Recommended dosages for each component are detailed in the table below.

Cell type	HEK-293/ HEK-293F	CHO-S/ CHO-K1
Cell density at transfection	2-3×10^6 cells/ml	5-6×10^6 cells/ml
Dosage of PEI Buffer	1/20 of the cell culture volume	1/20 of the cell culture volume
Dosage of plasmid DNA	0.4-0.8 μg/10^6 cells	0.3-0.8 μg/10^6 cells
PEI to DNA ratio	PEI:DNA=2:1-4:1	PEI:DNA=2:1-5:1

(2) Transfection steps:

Taking the transfection of 100 ml HEK-293 cells with a cell density of 3×10^6 cells/ml or CHO-S cells with a 6×10^6 cells/ml as an example. 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 cell density should reach 2-3×10^6 cells/mL (for HEK-293 cells) or 5-6×10^6 cells/mL (for CHO-S cells). 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 all the above matters are confirmed to be correct, the transfection of suspension cells can be optimized by adjusting the amount of plasmid DNA added, the ratio of PEI to plasmid DNA, and the cell density before transfection. It is recommended that the amount of plasmid DNA is 0.3–0.8 μg/10^6 cells; The optimized ratio of DNA and PEI is 1:2-1:5; The cell density before transfection is 2-8×10^6 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

Recommended amounts of plasmid DNA and PEI for transfection of suspension cells at various volumes (for reference only)

Cell Type	Volume (ml)	Cell Density (10^6 cells/ml)	DNA (μg)	PEI (μl)	PEI Buffer (ml)
HEK-293/ HEK-293F	30	3	40.5	81-162	1.5
HEK-293/ HEK-293F	100	3	135	270-540	5.0
HEK-293/ HEK-293F	400	3	540	1080-2160	20
CHO-S/ CHO-K1	30	6	72	144-360	1.5
CHO-S/ CHO-K1	100	6	240	480-1200	5.0
CHO-S/ CHO-K1	400	6	960	1920-4800	20

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