MediRes Corp.

USER GUIDE **G1802**

PEI 40K Transfection Reagent

Product #: G1802
Quantity: 5 x 1 mL

Product Description

- Plasmid DNA transfection suitable for cells, with low cytotoxicity, high transfection efficiency, and low cost.
- This transfection reagent contains polyethyleneimine (PEI) covalently bound to human transferrin, providing a highly specific DNA transformation method known as transferrinfection.
- Transferrinfection is a high-efficiency nucleic acid delivery system based on transferrin receptor-mediated endocytosis. In the PEI-transferrinfection system, the gene transfer efficiency of PEI/DNA complexes are combined with the specific mechanism of receptor-mediated endocytosis via the transferrin receptor.
    - PEI mediates efficient gene transfer into a variety of cells.
    - 30 – 1,000-fold enhanced transfection efficiency depending on the cell line.
    - Gentle DNA transfection method and employs physiological uptake mechanisms.
    - Transfection efficacy depends on the cell type, the level of surface transferrin receptor expression.
        - Very high transfection rates have been shown for the tested tumor cell lines B16F10 melanoma, Neuro 2A neuroblastoma, and a variety of primary human melanoma cell lines.
        - In other established cell lines, such as HeLa, CHO, Jurkat, K562, HepG2, and COS, the PEI-Transferrinfection works with high efficiency, excellent reproducibility and with the advantage of being an extremely gentle procedure.
- The concentration of PEI 40K, the main component of this product, is 1 mg/ml.

Storage

- Store at -20^{0}C for long-term storage (avoid repeated freeze-thaw cycles).
- Can be stored at 4^{0}C for up to 3 months.

Protocol

1. Preparation of adherent cells (protocol provided for one-well of 6-well plate; refer to table below for other vessel types):
    A. The day before transfection, plate cells at an appropriate density so 70 – 85% confluent on the day of transfection.
    B. Before transfection, remove media and wash cells with PBS before replacing with 1.8 mL of Opti-MEM (can also use basal medium without serum or low serum (below 5%)).
    C. Placed the cells back in the incubator and proceed with preparation of transfection complexes (Step 3).

2. Preparation of suspension cells:
    A. On the day of transfection, centrifuge an appropriate number of cells to remove the medium and resuspended with Opti-MEM (can also use basal medium without serum or low serum (below 5%)).
    B. Placed the cells back in the incubator and proceed with preparation of transfection complexes (Step 3).

3. Transfection complexes were prepared:
    A. Preparation of **Solution A**: 100 µL of Opti-Mem (or serum-free base medium) and 2 µg of plasmid DNA. Mix well while injecting air into solution using pipette.
    B. Preparation of **Solution B**: 100 µL of Opti-Mem (or serum-free medium) and 6-8 µL of PEI 40 K Transfection reagent. Mix well while injecting air into solution using pipette.

4. Slowly add **Solution B** to **Solution A.** Mix well while injecting air into solution using pipette.

5. Incubate mixed solution at room temperature for 15 min to form DNA-PEI transfection complex.

6. Cell transfection: add the transfection complex to the cells dropwise while distributing evenly. Swivel the cell culture flask to ensure even distribution of the cell transfection reagent.

7. Place the cells in the incubator.

8. After 4 – 6 h, replace with complete media.

Note: amount of DNA, PEI, and cell numbers need to be optimized for best results.

Vessel Type	Growth area (cm^2)	Number of inoculated cells	DNA content (μg)	PEI (μL)	Transfection complex volume (μL)	Total volume (mL)
96-well plates	0.3	$(2\text{-}4)\times10^4$	0.1	0.3-0.4	10	0.1
24-well plates	1.9	$(1.2\text{-}2.4)\times10^5$	0.5-1	1.5-4	50	0.5
12-well plates	3.8	$(2.4\text{-}4.8)\times10^5$	1-2	3-8	100	1
6-well plates	9.5	$(6\text{-}10)\times10^5$	2-4	6-16	200	2
10 cm culture dish	55	$(4\text{-}6)\times10^6$	12-24	36-96	1000	12
T75 culture flask	75	$(6\text{-}10)\times10^6$	18-36	54-144	1000	15