

Polyethylenimine-Transferrinfection (Tf-PEI) Kit

Catalog Number BMS1003/a and BMS1003

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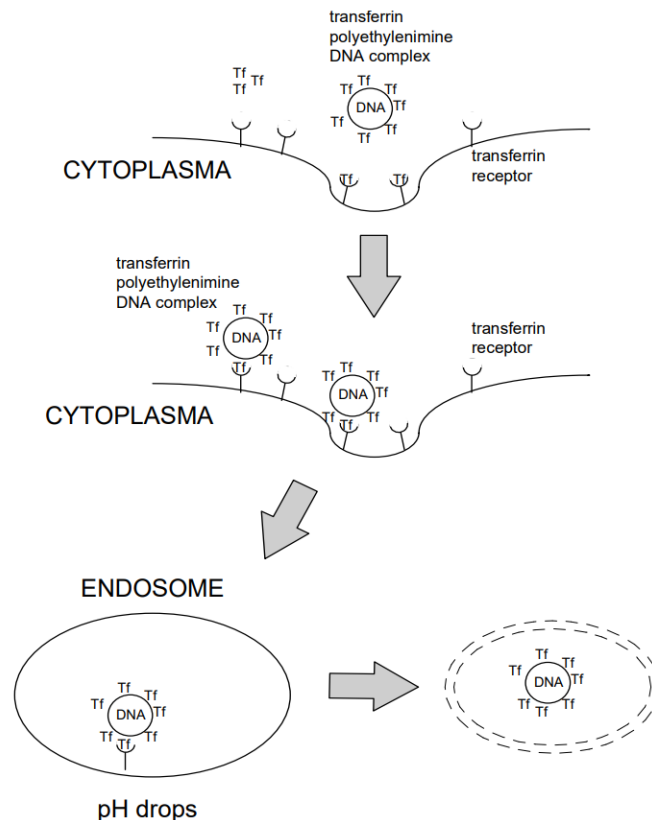
WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

Transfection of DNA into eukaryotic cells is a common method to study biological mechanisms. A major goal is the efficient and specific delivery of genes into the desired target cells. Although a wide panel of techniques and vectors (viral and nonviral) have been developed that work with variable efficiency, most vectors lack a target cell specificity. Nonviral vectors are attractive because of their ease of manipulation, safety, and high flexibility in the size of the delivered transgene. The Transferrinfection is a high-efficiency nucleic acid delivery system based on transferrin receptor-mediated endocytosis to carry DNA into cells. Furthermore it was shown that the cationic polymer polyethylenimine (PEI) mediates efficient gene transfer into a variety of cells. In the Polyethylenimine-Transferrinfection system, the gene transfer efficiency of PEI/DNA complexes are combined with the specific mechanism of receptor-mediated endocytosis via Transferrin receptor.

The method results in a 30–1,000-fold enhanced transfection efficiency depending on the cell line. It is an extremely gentle DNA transfection method that employs physiological uptake mechanisms of the cell. 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 efficiencies, excellent reproducibility and with the advantage of being an extremely gentle procedure.

Human transferrin is covalently linked to a polycationic carrier (Polyethylenimine/PEI) with intrinsic/ inherent endosomal activity. PEI has the capacity to condense DNA and deliver it into cells, presumably by adsorptive endocytosis. Furthermore, PEI is only partially protonated at physiological pH. Upon acidification within the endosome/ lysosome, PEI presumably acts as proton sponge with the protonation triggering chloride influx, osmotic swelling, and destabilization of the endosomal/ lysosomal vesicle enabling the release of the DNA into the cytosol. As a result, efficient gene transfer is obtained without the need for additional endosome destabilizing agents. The modified, PEI-conjugated, transferrin molecules maintain their ability to bind their cognate receptor and to mediate efficient iron transport into the cell. Transferrin receptor-mediated endocytosis occurs followed by the expression of the imported DNA (see Fig. 1). Deferrioxamine, a cell permeable iron chelator, increases the transferrin receptor density on the cell surface thus further enhancing gene delivery to the cell.



Contents and storage

Component	Quantity	Storage
Transferrin-Polyethylenimine Conjugate, lyophilized (1 mg/mL upon reconstitution; iron incorporated)	1 vial (BMS1003/a) 2 vials (BMS1003)	Store at 2–8°C.
Deferrioxamine, lyophilized (50 mM upon reconstitution)	1 vial	
HBS Buffer Concentrate (20X) (400 mM Hepes, pH 7.3; 3 M NaCl)	1 vial (1 mL)	

Prepare reagents

Transferrin-Polyethylenimine	Add 120 µL of distilled, sterile water to the vial containing iron-corporated Transferrin-Polyethylenimine. Ensure complete solubilization. Aliquot and freeze at -20°C.
Deferrioxamine	Add 500 µL of distilled, sterile water to the vial containing deferrioxamine. Ensure complete solubilization. The solution obtained is a 1,000X stock solution (50 mM). Aliquot and freeze at -20°C.
HBS-Buffer	Add contents of HBS-Buffer concentrate (1.0 mL) to 19 mL of distilled, sterile water and mix gently. Store at 2° to 8°C. If crystals have formed, warm gently until they have completely dissolved.

Protocol

The following protocol is calculated for the Transferrinfection of $2.5\text{--}5 \times 10^5$ cells plated in a 24-well cluster plate in 1.5 mL of medium. It can accordingly be adjusted to any other cell number, medium volume, and tissue culture plate.

• Prepare cells

Method	Description
Suspension cells	12 to 20 hrs before Transferrinfection, exponentially growing suspension cultures are collected by centrifugation and suspended in fresh medium (with serum) containing 50 µM deferrioxamine (from 1,000X stock solution). As an option before Transferrinfection, the cells may be collected and resuspended in fresh medium (with or without) serum containing 50 µM deferrioxamine.
Adherent cells	The cells are plated one day before Transferrinfection with medium containing 50 µM deferrioxamine (from 1,000X stock solution) and serum. As an option directly before transfection, the medium may be exchanged with fresh medium (with or without serum) containing 50 µM deferrioxamine. At this step, cells should be 50–70% confluent.

• Formation of transferrin-polyethylenimine conjugate/DNA complex

- Dilute purified DNA to a final concentration of 40 µg/mL in 1X HBS buffer.
- Prepare transfection cocktail (500 µL) according to the table:

N/P Ratio ^[1]	TfPEI (1 mg/mL)	DNA (40 µg/mL)	1X HBS
3.6	4.5 µL	250 µL	245.5 µL
4.0	5.0 µL	250 µL	245.0 µL
4.8	6.0 µL	250 µL	244.0 µL
5.2	6.5 µL	250 µL	243.5 µL
6.0	7.5 µL	250 µL	242.5 µL

^[1] PEI Nitrogen to DNA Phosphate groups

- Mix cocktail thoroughly by repeated aspiration and ejection for ~30 times. The ratios (N/P) may be crucial for the transfection efficiency. N/P ratios of at least 3.6 were shown to be necessary for successful gene transfer. For a number of cell lines, an N/P ratio of 4.8 to 6 were found to be optimal for gene transfer. However, to achieve optimal results, the individual optimum has to be titrated for each cell line/ DNA combination.

- Incubate transfection cocktail for 20 mins at room temperature before adding to the cells.

• Transferrinfection of transferrin-polyethylenimine/DNA complex

- Add 1.5 mL of fresh medium containing 50 µM deferrioxamine to the cells as indicated above.
- Add 500 µL of transfection cocktail to the cells plus medium.
- Incubate at 37°C for 4 hours.
- Remove the transfection medium, optionally wash cells once with prewarmed medium, and incubate in medium with the usual amount of serum.

Additional information

• Addition of serum

The Transferrinfection method can be done in the presence or absence of heat-inactivated fetal calf serum (FCS). Different batches of FCS might influence the transfection efficiency. Decreasing (or omitting) FCS during transfection will increase transfection efficacy, but may also result in increased toxicity.

• Addition of Deferrioxamine

This Transferrinfection method can be done in the presence or absence of Deferrioxamine, with Deferrioxamine increasing transfection efficacy in some cell lines. In most cases, treatment with Deferrioxamine is not necessary for efficient transfection.

• Quality of DNA preparation

The DNA quality is a critical parameter for Transferrinfection. The DNA transfected should be purified by cesium chloride gradients or commercial purification columns and should not be contaminated with RNA because RNA competes for complex formation of transferrin-polyethylenimine conjugate. Importantly, DNA has to be free of endotoxin because endotoxin was shown to increase toxicity and decrease efficacy of transfection procedure.

Limited product warranty

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Revision	Date	Description
A.0 (30)	10 March 2021	New manual.

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