

PEI-Transferrinfection Kit

BMS1003-a, BMS1003

A new method for receptor mediated polyethylenimine enhanced transfection of eukaryotic cells.

The Kit is suitable for:

- approx. 16 Transferrinfections in 24 well tissue plates (BMS1003/a) /
- approx. 32 Transferrinfections in 24 well tissue plates (BMS1003) /

1. Introduction:

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-Transferrinfectionsystem 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 - 1000-fold enhanced transfection efficiency depending on the cell line. It is an extremely gentle DNA transfection method which 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.

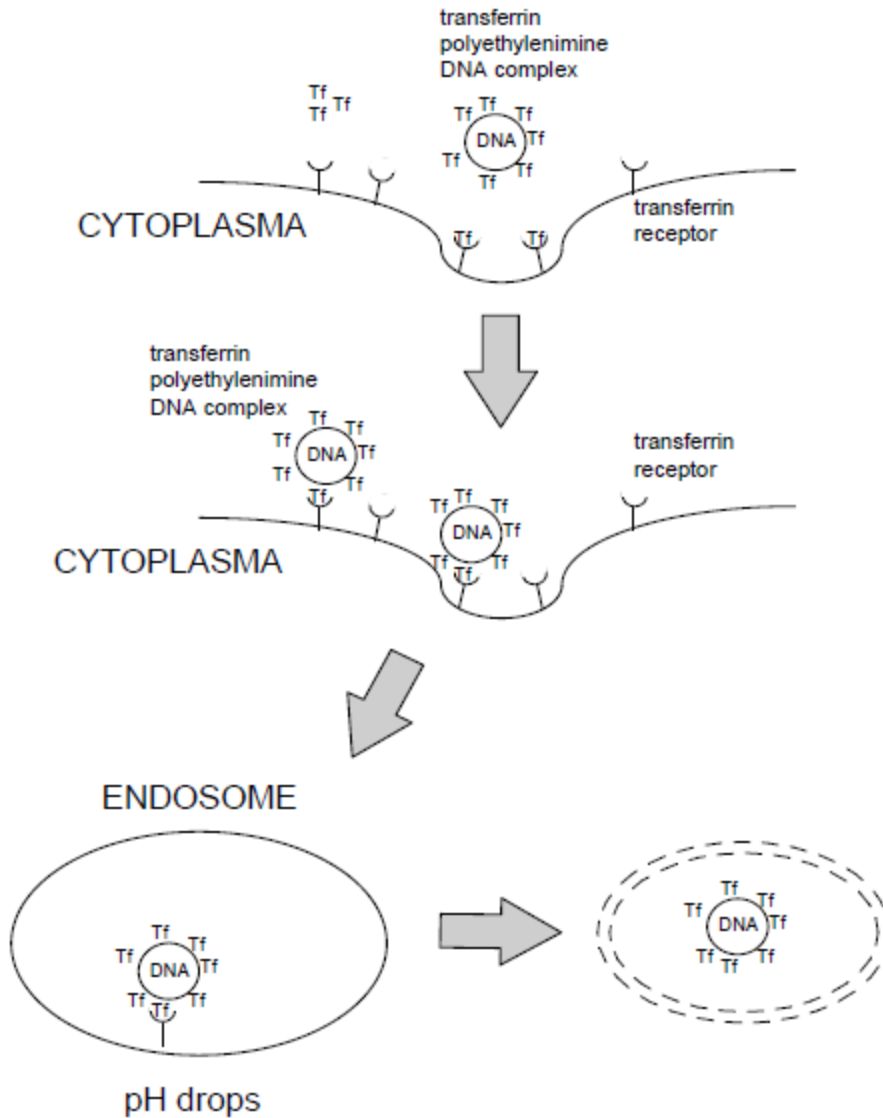
2. Principle:

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.

Figure 1)



3. Reagents provided:

- 1 (BMS1003/a) or 2 (BMS1003) vials Transferrin-Polyethylenimine-conjugate, lyophilized 1 mg/ml upon reconstitution; iron incorporated
- 1 vial Deferrioxamine; lyophilized, 50 mM upon reconstitution
- 1 vial (1 ml) HBS buffer concentrate (20 x) (400 mM Hepes, pH 7.3, 3M NaCl)

4. Preparation of reagents:

A) 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.

B) Deferrioxamine

Add 500 µl of distilled, sterile water to the vial containing deferrioxamine. Ensure complete solubilization. The solution obtained is a 1000x stock solution (50 mM). Aliquot and freeze at -20°C.

C) HBS-Buffer

Add contents of HBS-Buffer concentrate (1.0 ml) to 19 ml of distilled, sterile water and mix gentle. Store at 2° to 8°C. If crystals have formed, warm gently until they have completely dissolved.

5. Test Protocol:

a) Preparation of cells

1) Suspension cultures

12 to 20 h before Transferrinfection exponentially growing suspension cultures are collected by centrifugation and suspended in fresh medium (with serum) containing 50 μ M deferrioxamine (from 1000x stock solution). Furthermore, directly before Transferrinfection optionally the cells may be collected, and resuspended in fresh medium (with or without) serum containing 50 μ M deferrioxamine.

2) Adherent cells

The cells are plated one day before Transferrinfection with medium containing 50 μ M deferrioxamine (from 1000x stock solution) and serum. Directly before transfection optionally the medium may be exchanged by fresh medium (with or without serum) containing 50 μ M deferrioxamine. At this step cells should be 50 to 70% confluent.

The following protocol is calculated for the Transferrinfection of 2.5×10^5 cells plated in a 24 well cluster plate in 1.5 ml medium.

It can accordingly be adjusted to any other cell number, medium volume, and tissue culture plate.

b) Formation of transferrin-polyethylenimine-conjugate / DNA complex

- Dilute purified DNA to a final concentration of 40 μ g/ml in 1x HBS buffer (4.C)
- Prepare transfection cocktail (500 μ l) according to the following table:

N/P ratio*	TfPEI (1 mg/ml)	DNA (40 μ g/ml)	1 x HBS
3.6	4.5 μ l	250 μ l	245.5 μ l
4.0	5.0 μ l	250 μ l	245.0 μ l
4.8	6.0 μ l	250 μ l	244.0 μ l
5.2	6.5 μ l	250 μ l	243.5 μ l
6.0	7.5 μ l	250 μ l	242.5 μ l

*PEI Nitrogen to DNA Phosphate groups (N/P)

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

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

c) Transferrinfection of transferrin-polyethylenimine/ DNA complex

- Add 1.5 ml fresh medium containing 50 μ M Deferrioxamine to the cells as indicated above (5.a Preparation of cells)
- Add 500 μ l 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.

6. General considerations:

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, since RNA competes for complex formation of transferrin-polyethylenimine conjugate. Importantly, DNA has to be free of endotoxin since endotoxin was shown to increase toxicity and decrease efficacy of transfection procedure.

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Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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