DEAE-Dextran Transfection Kit

Product Code DE-DEX
Technical Bulletin No. MB 335

TECHNICAL BULLETIN

Product Description
DEAE-dextran transfection is a method for the introduction of DNA into eukaryotic cells. DEAE-dextran mediated transfection of MK cells with SV40 DNA was first described more than 20 years ago. In general, DEAE-dextran mediated transfection is successful for transient, but not stable transfection of cells. At higher DEAE-dextran concentrations, the exposure time to cells can be shortened in order to minimize cell death. DEAE-dextran facilitates DNA binding to cell membranes and entry of the DNA into the cell via endocytosis. As DEAE-dextran is toxic to cells, transfection conditions for individual cell lines may require careful optimization for both DEAE-dextran concentration and exposure times. DEAE-dextran is suitable for transfection of adherent as well as suspension cell lines.

Components
This kit allows for 60 transfections on 60 mm plates or 40 transfections on 100 mm plates. The reagents supplied in this kit are 0.2 µm filtered.

- DEAE-Dextran Solution, 20 ml
  Product Code D 6405
  Solution in phosphate buffered saline at 10 mg/ml

- 10x Phosphate Buffered Saline Solution 20 ml
  Product Code P 6437
  (10x PBS-TR), For Transfection Only

- 8 mM Chloroquine Solution in PBS, 2.5 ml
  Product Code C 7427

Reagents and Equipment Required but Not Provided
(Product Codes are provided where appropriate.)
- 1x Trypsin-EDTA Solution, Product Code T 3924
- Water, Molecular Biology Reagent, Product Code W 4502
- 100 mm culture plates, Product Code C 6796
- Cells for use in transfection

Optional Reagents Not Provided
(Product Codes are provided where appropriate.)
- DMSO, Product Code D 8418
- Glycerol, Product Code G 5516
- Tris-EDTA Buffer (TE), Product Code T 9285

Precautions and Disclaimer
The DEAE-Dextran Transfection Kit is for laboratory use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
Store all components at 2–8 °C.

Procedure
Two different procedures are described. The first procedure is a standard protocol, which includes a concurrent exposure of cells to DEAE-dextran and DNA. The second procedure involves pretreatment of the cells with DEAE-dextran and is a modification of a published procedure. It offers the advantage of a limited DEAE-dextran exposure, followed by a longer DNA incubation, allowing maximal DNA uptake. The best procedure for a particular cell line should be determined experimentally. For some cell lines, chloroquine dramatically increases transfection efficiencies, for others it has a minimal effect or it may be cytotoxic. The optimal amount of DNA to use for transfection will vary with the cell line and type of reporter construct being used. Generally, 2-6 µg of DNA will be sufficient for a 60 mm plate and 4-10 µg will be sufficient for a 100 mm plate.
Factors Affecting Gene Transfers

Transfection efficiencies can be increased in many cell types by additional treatments after the primary exposure of the cells to DEAE-dextran and DNA. The most effective and routinely used agents are glycerol, dimethyl sulfoxide (DMSO), chloroquine, and sodium butyrate. Since each of these chemicals is toxic to cells, the conditions for transfection of individual cell types must be carefully optimized for reagent concentration and exposure time.

Glycerol and DMSO solutions are applied to cells after the DNA is in association with the cells. The glycerol or DMSO solution [usually 10-20% (v/v) in Hank's Balanced Salt Solution (HBSS)] is applied to the cells for a period of 30 seconds to several minutes and then removed from the cells. The exact mechanism of action is unknown, but these treatments may modify cell membrane structure to enhance uptake of DNA.

Chloroquine, which is generally applied to the cells along with the DNA, appears to enhance transfection efficiency by binding to the DNA and inhibiting its degradation by lysosomes.

A. Preparation of DNA for Transfection

Supercoiled plasmid DNA is efficiently expressed following DEAE-dextran mediated transfections. The plasmid DNA to be used in the transfection should be free of protein, RNA, and chemical contamination. Ethanol-precipitated DNA should be resuspended in a sterile solution such as TE buffer to a final concentration of 0.2-1 mg/ml.

B. Standard DEAE-Dextran Procedure

1. Plating of the cells for the transfection experiment: Plate cells the day before the transfection experiment. The plating density for any particular cell line will depend on how quickly the cells divide. The cells should be 30-60% confluent the day of the transfection. An optimal plating density produces a nearly confluent plate when the cells are harvested or split into selective media, which is usually about 48 hours after the transfection. A general guideline is to plate about 8 x 10^5 cells per 100 mm culture plate. Scale down the number of cells proportionately for the 60 mm plate.

2. Prepare the Wash Solution of either 1x Phosphate Buffered Saline (PBS) or 1x Hanks' Balanced Salt Solution (HBSS) and warm it to 37 °C. Add 10 ml of Wash Solution for each 60 mm plate (20 ml for each 100 mm plate). Also, warm the DEAE-Dextran Solution to 37 °C.

3. Dilute the 10x PBS-TR stock solution 10-fold with sterile water. A 60 mm plate will need approximately 0.4 ml of 1x PBS-TR (0.6 ml per 100 mm plate).

4. Prepare the transfection solutions:
   - For a 60 mm plate: Using a sterile tube, dilute the DNA to a final volume of 326 µl in 1x PBS-TR. Add 17 µl of the DEAE-Dextran Solution and mix by gently tapping the tube.
   - For a 100 mm plate: Using a sterile tube, dilute the DNA to a final volume of 540 µl in 1x PBS-TR. Add 28 µl of the DEAE-Dextran Solution and mix by gently tapping the tube.

5. Remove the medium from the cells. Wash the cells twice with 5 ml of the Wash Solution for a 60 mm plate. Wash twice with 10 ml for a 100 mm plate.

6. Add the DNA/DEAE-dextran mixture and disperse it evenly over the cells. The final concentration of DEAE-dextran in the 1x PBS-TR is approximately 0.5 mg/ml.

7. Incubate the plates at 37 °C for 30 minutes. Rock the plates occasionally to keep the cells moist.

8. Gently add 3.5 ml of cell growth medium for each 60 mm plate (6 ml of medium for each 100 mm plate). Incubate up to 2.5 hours at 37 °C or until cytotoxicity is apparent, then gently change the medium or follow with a DMSO shock.
   - Optional: Add 35 µl of 8 mM chloroquine per 60 mm plate (60 µl per 100 mm plate) along with the medium during the 2.5 hour incubation step. If chloroquine is added, the culture medium must be replaced after 4 hours or earlier, if signs of cytotoxicity are apparent. The time that the cells are exposed to chloroquine must be empirically determined for each cell line.

9. Generally, cells may be harvested 48-72 hours after the transfection.
C. DEAE-Dextran Pretreatment Procedure
1. Plate the cells the day before the transfection experiment as described in step 1 of the Standard DEAE-Dextran Procedure.

2. Prepare the Wash Solution of either 1x Phosphate Buffered Saline (PBS) or 1x Hanks’ Balanced Salt Solution (HBSS).

3. Dilute the 10x PBS-TR stock solution 10-fold with sterile water. A 60 mm plate will need 3 ml of 1x PBS-TR (5 ml per 100 mm plate).

4. Prepare the transfection solutions:
   - Dilute the DEAE-Dextran Solution 10-fold with the 1x PBS-TR. A 60 mm plate will need 2 ml of Diluted DEAE-Dextran Solution (4 ml per 100 mm plate).
   - Dilute the DNA in 1x PBS-TR to a final volume of 325 µl for a 60 mm plate (540 µl for a 100 mm plate).

5. Remove the medium from the cells. Add 5 ml of sterile Wash Solution for 60 mm plates (10 ml for 100 mm plates). Incubate for 15 minutes at room temperature.

6. Remove the Wash Solution from the cells. Add 2 ml of the Diluted DEAE-Dextran Solution per 60 mm plate (4 ml per 100 mm plate). Incubate for 9 minutes at room temperature.

7. Remove the Diluted DEAE-Dextran Solution. Very gently, wash the cells twice with 5 ml of Wash Solution per 60 mm plate (10 ml per 100 mm plate). Be careful not to dislodge the cells, which may begin to detach after exposure to DEAE-dextran.

8. Remove the final wash. Add the diluted DNA in 1x PBS-TR and disperse it evenly over the cells. Incubate for 30 minutes in a 37 °C CO₂ incubator. Rock the plates occasionally to keep the cells moist.

9. Add 3.5 ml of cell growth medium per 60 mm plate (6 ml of cell growth medium per 100 mm plate).
   - Optional: Add 35 µl of 8 mM chloroquine per 60 mm plate (60 µl per 100 mm plate) together with the medium. If chloroquine is added, the culture medium must be replaced after 4 hours or earlier, if signs of cytotoxicity are evident. The time that the cells are exposed to chloroquine should be determined empirically for each cell line.

10. Return the plates to a 37 °C CO₂ incubator.

11. Generally, the cells may be harvested 48-72 hours after transfection.
## Troubleshooting Guide

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<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
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<tbody>
<tr>
<td>No transfection or low transfection efficiency</td>
<td>Excessive cell death</td>
<td>Decrease the concentration of DEAE-dextran or shorten the time to which the cells are exposed to DEAE-dextran. Decrease the time of exposure to chloroquine. Certain types of cells that are very sensitive to DEAE-dextran toxicity, such as primary cell cultures, may require a higher cell concentration at the time of transfection. For some cell lines, lower concentrations of DNA can be used for standard DEAE-dextran transfections compared to calcium phosphate transfections. Establish a dose-response curve to determine the optimal DNA concentration to use.</td>
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<tr>
<td>Variable transfection efficiencies in replicate experiments</td>
<td>Cells are contaminated with mycoplasma.</td>
<td>Test cultures for mycoplasma contamination. Destroy contaminated cultures and start a new culture from a fresh stock.</td>
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<td>Suboptimal growth of cells</td>
<td></td>
<td>Transfection efficiency may decrease if cells have been passaged for many generations. Start a fresh culture from cell stocks that were frozen at an early passage. Some cells, particularly lymphocytes, will exhibit variability in transfection efficiency if they are left in culture beyond 1-2 weeks.</td>
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### References

### Related Products
- Calcium Phosphate Transfection Kit, Product Code CA-PHOS
- ESCORT™ Liposome Transfection Reagent, Product Code E 9770
- DOTAP, Product Code D 1163

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