

DEAE—葡聚糖法细胞转染*

Materials

Cells to be transfected and appropriate culture medium (e.g., complete DMEM;) with and without 10% FBS

100 mM (1000×) chloroquine diphosphate in PBS, filter-sterilized (store at 4°C)

Plasmid DNA(s), prepared by CsCl density-gradient centrifugation or affinity chromatography

TE buffer

10 mg/ml DEAE-dextran stock solution

10% (v/v) dimethyl sulfoxide (DMSO) in PBS, filter-sterilized (store up to 1 month at RT)

Phosphate-buffered saline (PBS)

Appropriate-sized tissue culture vessels (Table A)

Inverted microscope

Additional reagents and equipment for mammalian cell culture

Method

1. Plate cells at a density to achieve 50% to 75% confluence on the target day for transfection. For COS or CV1 cells, perform a 1:10 split 2 days prior to transfection.

The surface area of various cell culture vessels given in Table 9.2.1 can be used to determine how to split cells to the desired density.

Some cell types including many primary cells show particular sensitivity to the toxicity of DEAE-dextran. These cells should be plated at higher density or transfected after reaching near-confluence.

2. Determine the total volume of medium to be used in the transfection based on the number of culture vessels containing cells to be transfected and the volume per vessel shown in Table A. Make up this amount of medium (plus some excess) to contain 2.5% FBS by combining 1 part medium containing 10% FBS with 3 parts serum-free medium.

DEAE-dextran can precipitate in the presence of high medium protein, necessitating use of a low FCS concentration. Alternatively, NuSerum (Collaborative Research), which contains only ~30% serum, can be used at a final concentration of 10%.

3. Add 100 mM (1000×) chloroquine diphosphate stock solution to the 2.5%-FBS-containing transfection medium prepared in step 2 to achieve a final concentration of 100 μM. Warm transfection medium to 37°C.

Chloroquine is toxic to all cells, so exposure time should be limited to <4 hr. If longer transfection times are required for optimal transfection of a particular cell type, chloroquine should be added during the final hours of the transfection.

4. Dilute plasmid DNA in TE buffer or distilled water to between 1.0 and 0.1 μg/μl, depending on the quantity to be transfected. Add the DNA solution directly to the warmed transfection medium to a final concentration of 1.0 μg/ml.

DNA solution(s) should comprise <1% of the total volume of transfection medium, so that the concentration of medium components is not significantly altered. Optimal DNA concentration in the transfection medium may have to be determined experimentally.

Maintaining dilute stock DNA solutions for dedicated use in transfections reduces interexperimental

variation as well as the time required to set up transfection experiments.

5. Warm the 10 mg/ml DEAE-dextran stock solution to 37°C and mix thoroughly by inversion. Add to the DNA-supplemented transfection medium to a final concentration of 100 µg/ml DEAE-dextran and mix by inversion.

The order of addition to the transfection medium is critical. Adding plasmid DNA to medium that has already been supplemented with DEAE-dextran can result in precipitation, seen as a ropy white glob. Optimal DEAE-dextran concentration in the transfection may have to be determined experimentally.

6. Aspirate medium from the 50% to 70% confluent cell cultures (see step 1) and replace

with the appropriate volume of 37°C DEAE-dextran/DNA-supplemented transfection medium (see Table A). Incubate 4 hr.

Uniformity of transfection efficiency may be improved by placing culture vessels on a rocker platform within the incubator during the transfection to ensure even exposure of cells to DEAE-dextran/DNA in the medium and to avoid desiccation of cells in the center of the vessel. Optimal transfection time may have to be determined experimentally.

7. Examine cells with an inverted microscope.

Cells may appear granular, some cell nuclei may appear pyknotic, and some cell borders may be somewhat ragged. An efficient DEAE-dextran transfection is usually associated with 25% to 75% cell death.

8. Warm the 10% DMSO/PBS to 37°C. Aspirate the transfection medium, note the volume, and replace with 2 to 3 volumes of 37°C DMSO/PBS. Incubate at room temperature for >2 but <10 min. Aspirate the DMSO/PBS and wash the cell layer with a volume of PBS equal to the amount of DMSO/PBS removed. Aspirate and replace with a standard amount of complete medium containing 10% FBS.

Loss of firm cellular anchorage to the culture vessel may occur. Medium exchange and cell washing should therefore involve careful aspiration and pipetting, perhaps by holding the tip of the pipet against a wall of the culture dish or well. It is sometimes advisable to omit the PBS wash (as in the experiment described in Alternate Protocol 1, step 8) and simply add the complete medium, then change the medium a second time several hours after the DMSO shock when cells have recovered and are more firmly adherent.

9. Continue incubating the cells and analyze at times appropriate to the bioassay or intended purpose of the experiment.

The onset and duration of expression of the transfected gene varies from one cell type to another, and especially with the expression vector used. It is advisable to perform a parallel transfection with a readily assayable reporter gene in the identical vector to assess the temporal features of expression. A reporter that is secreted by the cell into the culture medium, such as human growth hormone or secreted alkaline phosphatase, is ideal for this purpose, since aliquots of medium from a single transfection sample can be collected at serial time points. This parallel transfection can also be used in preliminary experiments to optimize transfection conditions.

REAGENTS AND SOLUTIONS

Complete DMEM

Dulbecco's modified Eagle medium, high-glucose formulation (e.g., Invitrogen), containing:

5%, 10%, or 20% (v/v) FBS (optional)

1% (v/v) nonessential amino acids

2 mM L-glutamine

100 U/ml penicillin

100 µg/ml streptomycin sulfate

Filter sterilize and store ≤1 month at 4°C

Throughout this manual, the percentage of serum (usually fetal bovine serum) used in a protocol step is indicated by a numeral hyphenated to the base medium name. Thus, "complete DMEM-10" indicates that 10% FBS is used. Absence of a numeral indicates that no serum is used. See Chapter 9 introduction for a full discussion concerning media preparation and use of serum (heat-inactivation, screening, commercial sources, etc.). DMEM containing 4500 mg/liter D-glucose can be obtained from Invitrogen. DMEM is also known as Dulbecco's minimum essential medium.

FBS (fetal bovine serum)

Thaw purchased fetal bovine serum (shipped on dry ice and kept frozen at -20°C until needed). Store 3 to 4 weeks at 4°C. If FBS is not to be used within this time, aseptically divide into smaller aliquots and refreeze until used. Store ≤1 year at -20°C

Repeated thawing and refreezing should be avoided as it may cause denaturation of the serum. In some cases, heat inactivation may be warranted (see Culture Medium Preparation).

To inactivate FBS, heat 30 to 60 min in a 56°C water bath. Alternatively, FBS may be inactivated through radiation treatment.

L-Glutamine, 0.2 M (100×)

Thaw frozen L-glutamine, aliquot aseptically into usable portions, then refreeze. For convenience, L-glutamine can be stored in 1-ml aliquots if 100-ml bottles of medium are used, and in 5-ml aliquots if 500-ml bottles are used. Store ≤1 year at -20°C.

Many laboratories supplement medium with 2 mM L-glutamine—1% (v/v) of 100× stock—just prior to use

TE (Tris/EDTA) buffer

10 mM Tris~Cl, pH 7.4, 7.5, or 8.0 (or other pH)

1 mM EDTA, pH 8.0

DEAE-dextran stock solution, 10 mg/ml (100×)

Make a 10 mg/ml stock solution of diethylaminoethyl (DEAE)-dextran (mol. wt. ~500,000 Da; Sigma) in tissue culture-quality PBS. Mix well, filter sterilize using an 0.22-µm filter, mix again, divide into aliquots, and store up to 3 months at 4°C.

Warm to 37°C and mix well by inversion immediately before each use.

PBS (phosphate-buffered saline):

10× stock solution, 1 liter:

80 g NaCl

2 g KCl

Working solution, pH ~7.3:

137 mM NaCl

2.7 mM KCl

11.5 g Na₂HPO₄·7H₂O
2 g KH₂PO₄

4.3 mM Na₂HPO₄·7H₂O
1.4 mM KH₂PO₄

Table A Surface Areas of Commonly Used Tissue Culture Vessels and Corresponding Appropriate DEAE-Dextran Transfection Medium Volumes

Vessel	Area (cm ²)	Appropriate vol. DEAE-dextran medium ^a (ml)
T175 flask	175	
T150 flask	150	
T75 flask	75	
T25 flask	25	
150- mm dish	148 ^b	10
100- mm dish	55 ^b	4
60- mm dish	21 ^b	2
35- mm dish	8 ^b	1
6- well plate(35-mm wells)	9.4 ^b	1
12- well plate(22-mm wells)	3.8 ^b	0.5
24- well plate(15.5-mm wells)	1.9 ^b	0.25

^aThese volumes are roughly a linear function of vessel surface area. To ensure that cells are completely covered by medium during the transfection, small wells require proportionately larger volumes due to annular sequestration of medium because of surface tension at the periphery.

^bCostar; other manufacturer products may deviate slightly.

*: 出自 Current Protocols in Molecular Biology [B], Wiley Online Library, 2007