磷酸钙法细胞转染*

Materials

Exponentially growing eukaryotic cells (e.g., HeLa, BALB/c 3T3, NIH 3T3, CHO, or rat embryo fibroblasts)
Complete medium (depending on cell line used)
CsCl-purified plasmid DNA (10 to 50 µg per transfection)
2.5 M $CaCl_2$
2× HEPES-buffered saline (HeBS;)
PBS
10-cm tissue culture dishes
15-ml conical tube
NOTE: All solutions and equipment coming into contact with cells must be sterile, and proper sterile technique should be used accordingly.
NOTE: All culture incubations are performed in a humidified 37°C, 5% CO_2 incubator unless otherwise specified.

Method

1. Split exponentially growing eukaryotic cells into 10-cm tissue culture dishes the day before transfection. Feed cells with 9.0 ml complete medium 2 to 4 hr prior to precipitation.

When transfecting adherent cells that double every 18 to 24 hr, a 1:15 split from a confluent dish generally works well. On the day of the transfection, it is important that cells are thoroughly separated on the dish, as the ability to take up DNA is related to the surface area of the cell exposed to the medium. Cells should be split in a manner that accomplishes this.

The desired density of cells on dishes to be transfected will vary with cell type and the reason for doing the transfection. The optimal density is that which produces a near confluent dish when the cells are harvested or split into selective medium.

2. Ethanol precipitate the DNA to be transfected and air dry the pellet by inverting the microcentrifuge tube on a fresh Kimwipe inside a tissue culture hood. Resuspend the pellet in 450 µl sterile water and add 50 µl of 2.5 M $CaCl_2$.

The amount of DNA that is optimal for transfection varies from 10 to 50 g per 10-cm plate, depending on the cell line to be transfected.

DNA to be transfected should be purified twice by CsCl gradient centrifugation.DNA can also be prepared using column procedures (UNIT 2.1B). Some column procedures produce DNA that does not transfect well, so column-purified DNA should be tested and compared to CsCl-purified DNA for transfection efficiency. Supercoiled DNA works well in transfections. Impurities in the DNA preparation can be deleterious to transfection efficiency. A description of how to optimize the amount of DNA to transfect and other parameters of calcium phosphate–mediated transfection is provided in the discussion of Optimization of Transfection.

Ethanol precipitation sterilizes the DNA to be transfected. For transfections that will be harvested within 3 to 4 days (transient analysis), this is not necessary. For transient experiments, many researchers make a 450-l aqueous solution containing the DNA directly, without ethanol

precipitation. If this is done, care should be taken to keep the amount of Tris in the solution to a minimum, as Tris may alter the pH of the precipitate and therefore reduce transfection efficiency.

3. Place 500 µl of 2× HeBS in a sterile 15-ml conical tube. Use a mechanical pipettor attached to a plugged 1- or 2-ml pipet to bubble the 2× HeBS and add the DNA/$CaCl_2$ solution dropwise with a Pasteur pipet (see Fig. 9.1.1). Immediately vortex the solution for 5 sec.

If no mechanical pipettor is available, the solution can be bubbled by blowing through rubber tubing that is attached to a pipet via a filter. The filter is necessary to maintain sterility. This does not give as reproducible results as the mechanical pipettor.

4. Allow precipitate to sit 20 min at room temperature.

5. Use a Pasteur pipet to distribute the precipitate evenly over a 10-cm plate of cells and gently agitate to mix precipitate and medium.

6. Incubate the cells 4 to 16 hr under standard growth conditions. Remove the medium. Wash cells twice with 5 ml PBS and feed cells with 10 ml complete medium.

The amount of time that the precipitate should be left on the cells will vary with cell type. For hardy cells such as HeLa, NIH 3T3, and BALB/c 3T3, the precipitate can be left on for 16 hr. Other cell types will not survive this amount of exposure to the precipitate.

See discussion of Optimization of Transfection for optimization of this step as well as for a discussion of how to determine whether glycerol shock is useful.

7. For transient analysis, harvest the cells at the desired time point.For stable transformation, allow the cells to double twice before plating in selective medium.

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

$CaCl_2$, 2.5 M:

183.7 g $CaCl_2$~$2H_2O$ (Sigma; tissue culture grade)
H_2O to 500 ml
Filter sterilize through a 0.45-µm nitrocellulose filter (Nalgene)
Store at −20°C in 10-ml aliquots

This solution can be frozen and thawed repeatedly.

PBS (phosphate-buffered saline):

10× stock solution, 1 liter:	*Working solution, pH ~7.3:*
80 g NaCl	137 mM NaCl
2 g KCl	2.7 mM KCl
11.5 g Na_2HPO_4~$7H_2O$	4.3 mM Na_2HPO_4~$7H_2O$
2 g KH_2PO_4	1.4 mM KH_2PO_4

HEPES-buffered saline (HeBS) solution, 2×

16.4 g NaCl (0.28 M final)
11.9 g HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; 0.05 M final)
0.21 g Na_2HPO_4 (1.5 mM final)
800 ml H_2O
Titrate to pH 7.05 with 5 N NaOH
Add H_2O to 1 liter
Filter sterilize through a 0.45-µm nitrocellulose filter
Test for transfection efficiency
Store at −20°C in 50-ml aliquots

An exact pH is extremely important for efficient transfection. The optimal pH range is 7.05 to 7.12.

There can be wide variability in the efficiency of transfection obtained between batches of 2× HeBS. Efficiency should be checked with each new batch. The 2× HeBS solution can be

rapidly tested by mixing 0.5 ml of 2× HeBS with 0.5 ml of 250 mM $CaCl_2$ and vortexing. A fine precipitate should develop that is readily visible in the microscope. Transfection efficiency must still be confirmed, but if the solution does not form a precipitate in this test, there is something wrong.

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