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Calcium PhosphateTransfection Kit

Catalog number K2780-01

Part no. 250067 MAN0000017 **Rev. Date** 11 August 2011

Kit Contents

Supplied reagents are sufficient to perform 1 control and 75 transfection reactions.

Component	Quantity
Tissue Culture Sterile Water	2 × 12 mL
2X Hepes Buffered Saline (HBS)	2 × 12 mL
2 M $CaCl_2$	3 × 1 mL
pcDNA™3.1/His/*lacZ*	20 µg

Storage

Upon receipt, store all components at room temperature.

Description

The Calcium Phosphate Transfection method for introducing DNA into mammalian cells is based on forming a calcium phosphate-DNA precipitate. Calcium phosphate facilitates the binding of the DNA to the cell surface. DNA then enters the cell by endocytosis. The method was first developed by Graham and van der Ebb (1) and was later modified by Wigler (2). The procedure is routinely used to transfect a wide variety of cell types for transient expression or for producing stable transformants. The DNA is mixed directly with a concentrated solution of $CaCl_2$, which is then added dropwise to a phosphate buffer to form a fine precipitate. Aeration of the phosphate buffer while adding the DNA-$CaCl_2$ solution helps to ensure that the precipitate that forms is as fine as possible, which is important because clumped DNA will not adhere to or enter the cell as efficiently.

Important Guidelines for Transfection

- Use a final $CaCl_2$ concentration of 60 mM for calcium phosphate transfections.
- The final volume of DNA-$CaCl_2$ should not exceed 1/10th of the volume of the media in which the cells are plated.
- Transfections can be carried out in either 60-mm or 100-mm dishes.
- Cells should be seeded at a density such that on the day of transfection they are no more than 50% confluent. The optimal seeding density produces a nearly confluent dish of cells when cells are harvested or split into selective media 48 hours after the transfection. This will vary for each cell line and is dependent upon the doubling time of the cell. In General, seed cells at a density of 5×10^5 for a 60-mm dish or $1 \times 10^6 - 2 \times 10^6$ for a 100-mm dish.
- Between 10 and 100 µg of DNA may be transfected.
- Transfection efficiencies may be increased in some cell lines when the transfection is followed by either a glycerol (3, 4) or dimethylsulphoxide [DMSO] (4–6) shock. These chemicals are toxic to cells; therefore, the conditions for individual cell types must be optimized. The exact mechanism is unknown but may involve chemical alteration of the cell membrane to facilitate DNA uptake.

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Standard Transfection Procedure

Day 1: Prepare Cultured Cells for Transfection

Plate cells in 100-mm or 60-mm dishes at the required density. Incubate the dishes overnight at 37°C in a humidified CO_2 incubator.

Day 2: Transfection

1. 3–4 hours prior to transfection, change the media on the dishes.
2. Transfection Mixture:

For a 100-mm dish with 10 mL of media:	For a 60-mm dish with 5 mL of media:
To a tube labeled A add:	To a tube labeled A add:
36 µL 2 M $CaCl_2$	18 µL 2 M $CaCl_2$
20 µg DNA	10 µg DNA
Volume to 300 µL with sterile H_2O.	Volume to 150 µl with sterile H_2O.
To a tube labeled B add:	To a tube labeled B add:
300 µL 2X Hepes Buffered Saline (HBS)	150 µL 2X Hepes Buffered Saline (HBS)

3. Using a pasteur pipette, slowly add solution A dropwise to solution B while bubbling air through solution B with another pipette. Continue until solution A is depleted. This is a slow process which should be done over 1–2 minutes. A fine precipitate should form.
4. Incubate at room temperature for 30 minutes.
5. Add the precipitate dropwise to the media to the cells in the 60-mm or 100-mm dish.
6. Incubate the cells overnight at 37°C in a humidified CO_2 incubator.

Day 3: Change Media and Optionally Shock Cells

1. Remove the media from the cells.
2. Wash the cells twice with 1X Phosphate Buffered Saline (PBS).
3. Depending on the cell line, perform a glycerol or DMSO shock:

   Glycerol Shock:
   a. Prepare a fresh 15% glycerol shock solution in 1X HBS.
   b. Wash the cells once with 1X PBS.
   c. Add 2 mL glycerol solution per 60-mm dish or 3 mL per 100-mm dish.
   d. Incubate the dish(es) at room temperature for exactly 2 minutes.
   e. Remove glycerol and wash once with 1X PBS. Proceed to step 4.

   DMSO Shock:
   a. Prepare a fresh stock of 10% DMSO in 1X PBS.
   b. Remove the media from the plate and add 2 mL DMSO solution per 60-mm dish or 3 mL per 100- mm dish.
   c. Incubate the dish at room temperature for exactly 2.5 minutes.
   d. Remove DMSO and add fresh media. Proceed to Step 4.
4. Add fresh media and incubate the cells at 37°C in a humidified CO_2 incubator for 24–48 hours.

Day 4 or 5: Harvest Cells

For transiently expressing your gene of interest, harvest the cells 48–72 hours post-transfection.

1. Remove the media and wash the dish with 5 mL of 1X PBS.
2. Remove and add 5 mL of fresh 1X PBS to the dish. Using a rubber police-man, scrape cells gently off the bottom of the dish.
3. Transfer cell suspension to a 15-mL Falcon tube and spin at 1500 rpm for 5 minutes.
4. Resuspend the cell pellet in 1 mL of 1X PBS, then transfer the cell suspension to a sterile eppendorf tube.
5. Spin the cell suspension for 5 minutes in a microcentrifuge.
6. Remove the supernatant and freeze pellets at –70°C until needed.

Selecting Stable Transformants

1. To generate stable long-term clones, identify individual clones in which the DNA has integrated into the chromosome. This can be accomplished by including a drug resistance marker in the transfected DNA and selecting with the appropriate drug (refer to references 7, 8 and 9 on page 4).
2. Determine the concentration of the drug used for selection, which will vary according to the antibiotic and the cell line used, before the experiment is undertaken.
3. Plot a kill-curve for your cell line of interest by measuring cell growth under increasing concentrations of the drug. The optimal concentration is one that kills the cells within 10–14 days.
4. Typically, you will maintain cells in non-selective media for 2–3 days post-transfection before trypsinizing and replating the cells in selective media.
5. Maintain the selection for 2–3 weeks with frequent media changes to eliminate dead cells. Continue until discreet colonies can be visualized.
6. Trypsinze individual colonies using cloning rings and transfer the colony to microtiter wells for subsequent propagation.

For a more detailed description of procedures used for selection of stable clones, refer to references 10 and 11, on page 4.

Control Vector

Control vector pcDNA™3.1/His/*lacZ* is included to allow you to:

- Detect the protein on a western blot with an anti-his antibody.
- Detect enzyme activity with a β-Gal detection kits, such as the β-Gal Assay Kit (Cat. no. K1455-01) and the NovaBright™ β-galactosidase Enzyme Reporter Gene Chemiluminescent Detection Kit for Mammalian Cells (Cat. no. N10563).
- Stably select with Geneticin.

References

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