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Product Information

Calcium Phosphate Transfection Kit
Product Number **CAPHOS**

Technical Bulletin

Introduction

Calcium phosphate transfection is a commonly used method for the introduction of DNA into eukaryotic cells. This technique has been used to obtain both transient[1] and stable[2] transfections in a wide variety of cell types. The procedure is based on slow mixing of HEPES-buffered saline containing sodium phosphate with a $CaCl_2$ solution containing the DNA. A DNA−calcium phosphate co-precipitate forms, which adheres to the cell surface and is taken up by the cell, presumably by endocytosis. Glycerol shock may increase the uptake of DNA in some cell types.

Reagents Provided

The reagents supplied in this kit are sterilized by 0.2 μm filter and aseptically filled.
This kit allows for either 80 transfections on 10 cm dishes, or 160 transfections on 6 cm dishes (~25 - 6 well plates), or 400 transfections on 3.5 cm dishes (~ 36 - 12 well plates).

The Calcium Phosphate Transfection Kit contains the following:

- 1 vial (5 mL) 2.5 M $CaCl_2$, Catalog Number C2052
- 1 vial (25 mL) Molecular Biology grade water, Catalog Number W4502
- 1 vial (25 mL) 2x HEPES Buffered Saline, pH 7.05 (2x HeBS), Catalog Number H1012
  50 mM HEPES, 280 mM NaCl, 1.5 mM Na_2HPO_4

Storage

All components should be stored at –20 °C
Allow all kit components to thaw and equilibrate to room temperature before use.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Procedure

The procedure stated below is designed for the transfection of CHO cells with 1 μg/μL pSV40-CAT plasmid (diluted in sterile molecular biology water). Culture cells in standard serum-containing or serum-free medium appropriate for the cell type. Antibiotics are not recommended. Use good aseptic technique and use only sterile materials.

DNA plasmids should be high-quality, ethanol-precipitated, resuspended in molecular biology grade water to a final concentration of 1 μg/μL

This protocol can be optimized for use with a wide variety of cell types. Seeding density, amount of DNA used, incubation time and glycerol shock can easily be varied to achieve higher expression and lower toxicity when needed.

<u>Day One: Plate Cells</u>
Plate the cells according to the following chart:

Culture Dish	Cell Plating Density
3.5 cm dish	2×10^5
6 cm dish	5×10^5
10 cm dish	$1 – 2 \times 10^6$

Day Two: Transfection

1. To prepare the cells for transfection, add fresh complete medium according to the chart below:

Culture Dish	Fresh complete medium added (mL)
3.5 cm dish	1
6 cm dish	2
10 cm dish	4

2. Two hours later, prepare two tubes with transfection reagents as follows:

Culture Dish	Tube A (mix gently)			Tube B
	$CaCl_2$ (µL)	H_2O (µL)	DNA (µL)	2x HeBS (µL)
3.5 cm dish	6	49	5	60
6 cm dish	15	123	12	150
10 cm dish	30	245	25	300

3. Bubble the 2x HeBS (tube B) using an automatic pipette pump attached to a 1 mL serological pipette fitted with a 200 µL pipette tip.
4. While bubbling the 2x HeBS (tube B), add contents of tube A (from step 2), dropwise.
5. Vortex for 2 – 4 seconds.
6. Allow the precipitate to sit undisturbed for 20 minutes.
7. Drop the solution evenly over the cell culture medium on the plate. Gently agitate the dish to distribute the precipitates evenly over the cells on the plate.
8. Incubate cells overnight (approximately 16 hours).

Day Three: Optional Glycerol Shock

1. Mix the following in a centrifuge tube:

Culture Dish	50% sterile glycerol (µL)	2x HeBS (µL)	H_2O (µL)
3.5 cm dish	100	250	150
6 cm dish	225	565	335
10 cm dish	450	1130	670

2. Remove medium from the dish and replace with glycerol solution. Incubate 2 minutes.
3. Remove glycerol solution and wash twice with PBS:

Culture Dish	Volume PBS for each wash (mL)
3.5 cm dish	2
6 cm dish	5
10 cm dish	10

Day Three: Change Medium

1. Following overnight incubation (or optional glycerol shock), aspirate medium (or PBS) and replace with complete medium.
2. Incubate the cells for 48 hours.
3. Collect and lyse the cells – they are ready to be used for other applications.

References

1. Graham, F.L. and Van der Eb, A.J., Virology, **52**, 456 (1973)
2. Wigler, M. *et al*., Cell **14**, 725 (1978)

Related Products

Catalog Number	Description
D8662	Phosphate Buffered Saline (PBS)
G5516	Glycerol
SIAL0790	Sigma® centrifuge tube
T6524	Microcentrifuge tube (non-sterile)
SIAL1010	Sigma seriological pipette (1 mL)
SIAL1020	Sigma seriological pipette (2 mL)
SIAL1050	Sigma seriological pipette (5 mL)
SIAL1100	Sigma seriological pipette (10 mL)
CLS4864	Corning Universal fit pipet tips (1 – 200 µL)
SIAL0506	Sigma 6 well cell culture plate
SIAL0512	Sigma 12 well cell culture plate
SIAL0165	Sigma 3.5 cm cell culture dish
SIAL0166	Sigma 6 cm cell culture dish
SIAL0167	Sigma 10 cm cell culture dish
D1163	DOTAP
L3287	Escort IV Transfection Reagent

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