Calcium Phosphate Transfection Kit
Product Number CAPHOS

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 \(\mu\)m 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\(_2\)HPO\(_4\)

Storage
All components should be stored at \(-20^{\circ}\)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 \(\mu\)g/\(\mu\)L 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 \(\mu\)g/\(\mu\)L.

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.

Day One: Plate Cells
Plate the cells according to the following chart:

<table>
<thead>
<tr>
<th>Culture Dish</th>
<th>Cell Plating Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5 cm dish</td>
<td>2 x 10(^5)</td>
</tr>
<tr>
<td>6 cm dish</td>
<td>5 x 10(^5)</td>
</tr>
<tr>
<td>10 cm dish</td>
<td>1 – 2 x 10(^6)</td>
</tr>
</tbody>
</table>
Day Two: Transfection
1. To prepare the cells for transfection, add fresh complete medium according to the chart below:

<table>
<thead>
<tr>
<th>Culture Dish</th>
<th>Fresh complete medium added (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5 cm dish</td>
<td>1</td>
</tr>
<tr>
<td>6 cm dish</td>
<td>2</td>
</tr>
<tr>
<td>10 cm dish</td>
<td>4</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Culture Dish</th>
<th>Tube A (mix gently)</th>
<th>Tube B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CaCl₂ (µL)</td>
<td>H₂O (µL)</td>
</tr>
<tr>
<td>3.5 cm dish</td>
<td>6</td>
<td>49</td>
</tr>
<tr>
<td>6 cm dish</td>
<td>15</td>
<td>123</td>
</tr>
<tr>
<td>10 cm dish</td>
<td>30</td>
<td>245</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>Culture Dish</th>
<th>50% sterile glycerol (µL)</th>
<th>2x HeBS (µL)</th>
<th>H₂O (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5 cm dish</td>
<td>100</td>
<td>250</td>
<td>150</td>
</tr>
<tr>
<td>6 cm dish</td>
<td>225</td>
<td>565</td>
<td>335</td>
</tr>
<tr>
<td>10 cm dish</td>
<td>450</td>
<td>1130</td>
<td>670</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Culture Dish</th>
<th>Volume PBS for each wash (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5 cm dish</td>
<td>2</td>
</tr>
<tr>
<td>6 cm dish</td>
<td>5</td>
</tr>
<tr>
<td>10 cm dish</td>
<td>10</td>
</tr>
</tbody>
</table>

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
2. Wigler, M. et al., Cell 14, 725 (1978)

Related Products

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