CalPhos™ Mammalian Transfection Kit User Manual

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I. Introduction

The ability to introduce exogenous DNA into cultured cells is a powerful tool for molecular and cell biologists. Of the many methods to introduce DNA into mammalian cell cultures, the calcium phosphate method is one of the most widely used because it is inexpensive, simple, and suitable for a range of different cell types (Ausubel et al.,1994; Graham & van der Eb, 1973). The CalPhos™ Mammalian Transfection Kit provides high-quality, pretested reagents suitable for both transient and stable transfections. The kit includes all the reagents necessary to perform 100 transfections in 10-cm plates, or 725 transfections in 35-mm plates.

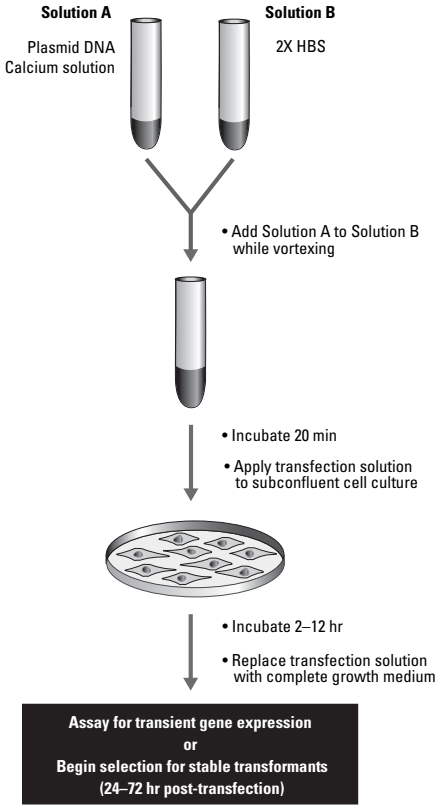

Figure 1. Flow chart for using the CalPhos™ Mammalian Transfection Kit.

II. List of Components

Store HBS at –20°C; store all other components at 4°C.
The following reagents are sufficient for 100 transfections in 10-cm plates or 725 transfections in 35-mm plates.

- 9 ml **2 M Calcium Solution**
- 2 x 35 ml **2X HEPES-Buffered Saline** (HBS)

We recommend dispensing this buffer into small aliquots, to be stored at –20°C. Avoid multiple freeze-thaw cycles. When an aliquot is in use, store it at 4°C for up to one week.

- 2 x 35 ml **Sterile H_2O**

III. Additional Materials Required

- **Cell culture plates or flasks**
- **Tubes** (12 x 75-mm sterile tubes)
- **Cell culture medium** (appropriate growth medium for mammalian cells in culture)
- **Fetal bovine serum, newborn calf serum, or equivalent** (to supplement the growth medium)
- **Phosphate buffered saline** (PBS; pH 7.4)

	Final Conc.	To prepare 2 L of solution
Na_2HPO_4	58 mM	16.5 g
NaH_2PO_4	17 mM	4.1 g
NaCl	68 mM	8.0 g

Dissolve the above components in 1.8 L of deionized H_2O. Adjust to pH 7.4 with 0.1 N NaOH. Add deionized H_2O to final volume of 2 L. Store at room temperature.

- **1X Trypsin/EDTA** (Life Technologies Cat. No. 25300-054)
- **Plasmid DNA**
  The DNA should be of high quality e.g., double CsCl-banded or column-purified DNA. Clontech offers many NucleoBond® Plasmid Purification Kits and cartridges which yield "transfection-grade" plasmids. See Related Products for more information.

IV. CalPhos™ Mammalian Transfection Protocol

The following protocol is designed for use with adherent cultures growing in **35-mm tissue-culture plates**. If you are using plates, wells, or flasks of a different size, adjust the volume of the transfection solution in accordance with your culture volume. See Appendix B for culture plate conversions.

All steps of the following protocol should be performed in a sterile tissue culture hood.

1. Plate the cells the day before the transfection experiment. The cells should be 50–80% confluent the day of transfection. Generally, we plate 4 x 10^5 cells/35-mm plate.
2. 0.5–3 hr prior to transfection, replace culture medium on plates to be transfected with 2 ml of fresh culture medium per 35-mm plate.
3. For each transfection, prepare Solution A and Solution B in separate sterile tubes.

   Solution A: add components in the following order:

2–4 µg	Plasmid DNA
	Sterile H_2O
12.4 µl	2 M Calcium Solution
100 µl	Total Volume

   Solution B: 100 µl 2X HBS

   Note: To reduce variability when transfecting multiple plates with the same plasmid DNA, prepare master solutions of Solutions A and B sufficient for all plates.
4. Carefully and slowly vortex Solution B while adding Solution A dropwise. (Alternatively, blow bubbles into Solution B with a 1-ml sterile pipette and an autopipettor while adding Solution A dropwise.)
5. Incubate the transfection solution at room temperature for 20 min.
6. Gently vortex transfection solution and then add solution dropwise to culture plate medium. (Add 200 µl of transfection solution per 35-mm plate.)
7. Gently move plates back and forth to distribute transfection solution evenly. (Do not rotate plates as this will concentrate transfection precipitate in the center of the well or plate.)
8. Incubate plates at 37°C for 2–12 hr in a CO_2 incubator.
9. Remove calcium phosphate-containing medium and wash cells with medium or 1X PBS.
10. Feed plate with 2 ml fresh complete growth medium and incubate at 37°C until needed for assay.
11. Assay for transient gene expression or start selection for stable transformants 24–72 hr post-transfection.

V. Troubleshooting Guide

A. Low Transfection Efficiency

- Poor precipitate formation

  Solution: Addition of the calcium/DNA (Solution A) to the 2X HBS (Solution B) should be performed dropwise and with continuous mixing. Adding Solution A too quickly or with too little mixing can result in a poor precipitate.

- Poor quality DNA

  Solution: The A_{260}/A_{280} ratio of the plasmid DNA should be ≥1.7.

- pH not optimal

  Solution: The pH of the HBS should be between 7.05 and 7.12. However, during prolonged storage, the pH of the solution may change; therefore, use the transfection kit within the shelf life indicated on the accompanying Product Analysis Certificate (PAC).

B. Variable Transfection Efficiency in Experiments

There will always be some variability in transfection efficiencies. We recommend performing transfections in triplicate to minimize the variability.

- Variable cell density

  Solution: Keep cell density constant after optimizing transfection procedures. Generally we use cultures that are 50–80% confluent at the time of transfection.

- Suboptimal cell growth

  Solution: Keep cells healthy in culture. Cells should be in mid-log phase growth when plated for transfection. Transfection efficiencies may decrease for cell lines that have been passaged for too many generations.

VI. References

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1994) In Current Protocols in Molecular Biology (Greene Publishing Associates and John Wiley & Sons, Inc., NY) Vol. 1, Ch. 9.

Freshney, R. I. (2000) Culture of Animal Cells, Fourth Edition (Wiley-Liss, NY).

VII. Related Products

For the latest and most complete listing of all Clontech products, please visit **www.clontech.com**

	Cat. No.
• Clonfectin™	631301
• Living Colors® Fluorescent Protein Reporter Vectors	many
• Tet-On® and Tet-Off® Expression Systems and Vectors	many
• Great EscAPe™ Secreted Alkaline Phosphatase (SEAP) Vectors and Kits	many
• β-galactosidase Vectors and Kits	many
• IRES Expression Vectors	many
• Retro-X™ Expression Vectors	many
• MATCHMAKER™ Mammalian Two-Hybrid Assay Kit	630301
• NucleoBond® Plasmid Kits, Mini Kit	635925 635926 635927
Midi Kit	635929 635930 635931
Maxi Kit	635933 635934 635935 635936
Mega Kit	635938
Giga Kit	635939
• NucleoBond® AX Tips	many

Appendix A: Optimization of Transfection

The efficiency of a mammalian cell transfection is primarily dependent on the host cell line used. Optimization of the transfection parameters for each cell type is crucial to obtaining consistently successful transfections. Therefore, for each cell type you plan to use, perform preliminary experiments to determine the optimal: 1) cell density; 2) amount and purity of DNA; and 3) transfection incubation time.

For the preliminary experiments, the host cell line can be transfected with a reporter expression vector, such as pCMVβ (Cat. No. 631719) or pSEAP2-Control Vector (Cat. No. 631717). The success of the transfection can then be estimated by assaying for β-galactosidase or secreted alkaline phosphatase. Once the transfection parameters have been optimized, they should be kept consistent from one experiment to the next to obtain reproducible results.

The following is a general guideline for optimizing the transfection parameters. To optimize transfection parameters, it is best to perform a series of small-scale transfections. This can be done conveniently in 12-well or 6-well plates.

To optimize cell density: keeping all other parameters constant, plate host cells in individual wells of a 6-well plate at varying densities (e.g., 5×10^4, 1×10^5, 2×10^5, 4×10^5, 8×10^5). 24–72 hours post-transfection, assay for reporter gene (SEAP or β-galactosidase) activity. Record results. Repeat the experiment once or twice to account for day-to-day variation. Choose the density with the highest reporter gene activity.

The other parameters can be optimized in much the same way. Hold all other variables constant while varying the parameter you are testing. Transfection incubations should be maximal at 2–16 hours using the CalPhos™ Mammalian Transfection Kit. You may want to try incubation times from 1–18 hours for optimization. After transfections have been optimized, scale-up or scale-down as necessary for the size of culture plate you are using (see Appendix B for a table of conversions).

Appendix B: Culture Plate Conversions

TABLE I. CULTURE PLATE CONVERSION

Size of Plate	Growth Area (cm^2)	Relative Area*	Recommended Volume
96 well	0.32	0.04 X	200 µl
24 well	1.88	0.25 X	500 µl
12 well	3.83	0.5 X	1.0 ml
6 well	9.4	1.2 X	2.0 ml
35 mm	**8.0**	**1.0 X**	**2.0 ml**
60 mm	21	2.6 X	5.0 ml
10 cm	55	7 X	10.0 ml
Flasks	25	3 X	5.0 ml
	75	9 X	12.0 ml

* Relative area is expressed as a factor of the growth area of a 35-mm culture plate.