Product: Calcium Phosphate Transfection System

Cat. No.: 18306-019

Lot No.: HHS704

Size: 75 Reactions

Storage Conditions: 4°C

Do Not Freeze

COMPONENT	AMOUNT	PART NO.
10X HBS [HEPES, NaCl]	4 X 1.25 ml	90136
1N NaOH	1.25 ml	90133
Calcium Solution	4 X 1.25 ml	90134
Phosphate Solution [mono- and di-basic sodium phosphate]	1.25 ml	90135
Transfection Qualified Water	100 ml	90137
Carrier DNA [salmon sperm DNA 10 mg/ml]	0.25 ml	90138

DESCRIPTION:

DNA can be introduced into tissue culture cells as a calcium phosphate-DNA complex (1,2). The transfected DNA can integrate into chromosomal DNA producing stable clones of altered genotype and phenotype. This system contains reagents sufficient for 75 transfections on 100-mm dishes. The components have been optimized for high efficiency transfection of nucleic acid sequences into cultured cells. Calcium phosphate-mediated transfection has been used for the introduction of DNA into a wide variety of cultured cell lines including:

Mouse:	L-cell	Rat:	FRTL-5	Monkey:	COS-1
	NIH3T3		BRL Cells		COS-7
	γ 2		GH3		CV-1
	γ AM		AR 42 J		Vero
	B78H1				
	AtT 20	Human:	HeLa	Hamster:	CHO
			C-4		
			293		

Quality Control Assays:

The Calcium Phosphate Transfection System is tested for the absence of bacterial and fungal contamination using thioglycolate and tryptic soy broth.

TK⁻ L cells are transfected with pTK-5 plasmid DNA and TK⁺ colonies are selected using HAT medium. NIH3T3 cells are also transfected with pRSVneo plasmid DNA and colonies are selected for resistance to 500 µg/ml G418.

Reference:

1. Wigler, M., Silverstein, S., Lee, L.S., Pellicer, A., Cheng, Y.C. and Axel, R. (1977) *Cell 11*, 223.
2. Graham, F.L. and Van der Eb, A.J. (1973) *Virology 52*, 456.

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Procedure for Stable Transfection:

1. Plate cells in a 100-mm tissue culture dish 24 h before transfection using the appropriate growth medium. The usual plating density is 1 x 10^6 cells/100-mm dish/10 mls complete medium. See Note 1.

2. Incubate the cells at 37°C in a humidified, 5% carbon dioxide environment.

3. Feed cells fresh, complete medium 3 h before transfection.

4. Prepare 1 ml of calcium phosphate-DNA suspension for each 100-mm plate of cells as follows. Use only sterile polypropylene tubes.

   a. Prepare 1X HBS (0.5 ml) fresh for each 100-mm dish. To prepare 1 ml of 1X HBS, add 0.885 ml Transfection Qualified Water and 0.1 ml 10X HBS to a sterile tube and mix well. Add 15 µl NaOH solution and mix well.
   b. Set up two sterile, polypropylene tubes for each DNA to be precipitated. See Note 2. Label the tubes 1 and 2. Add 0.5 ml 1X HBS and 10 µl Phosphate Solution to tube number 1. To tube number 2 add 0.43 ml Transfection Qualified Water minus volume "Y" (volume of carrier DNA, see below) and minus volume "Z" (volume of plasmid DNA, see below).

      DNA: The total amount of carrier DNA plus plasmid DNA should equal 20 µg.

      Carrier DNA: 20 µg carrier DNA minus "X" µg plasmid DNA = total µg of carrier DNA. The volume of carrier DNA is volume "Y" referred to above.

      Plasmid DNA: Amount added is at discretion of the investigator. The volume of plasmid DNA is volume "Z" referred to above.

      Genomic DNA: The total amount of genomic DNA used should be 30 µg. The volume of genomic DNA will take the place of volume "Y" for those using a marker plasmid with their genomic transfer.

   c. Add DNAs to tube number 2 and gently mix the DNAs into the water.
   d. Add 10 µl of Calcium Solution to tube number 2 and gently mix.
   e. Add 50 µl of Calcium Solution to tube number 2 and gently mix.
   f. Place a sterile 1-ml pipet into tube number 1 and gently bubble air through the solution so that it mixes slowly.
   g. Draw the contents of tube number 2 up into an appropriately sized, sterile pipet. Add slowly, dropwise to the gently bubbling and mixing solution in tube number 1. As the two solutions mix, they will appear milky and then form a white precipitate. Continue to bubble and add slowly until the pipet containing the contents of tube number 2 is empty.
   h. Stop the bubbling, cap the tube and check that a precipitate has formed by gently swirling the tube. Allow the suspension to sit at room temperature for 20 minutes.

5. Mix the precipitate well by pipetting or vortexing. Make sure that any large clumps that may have formed in the bottom of the tube are broken up and that the precipitate is evenly resuspended.

6. Add 1 ml of suspension to a 100-mm plate of cells containing 10 mls of complete medium. The suspension must be added slowly, dropwise while gently swirling the medium in the plate.

7. Return the plates to the incubator and leave the precipitate on for 10 to 24 h.

8. Remove the medium containing the precipitate and add fresh, complete medium. Leave this medium on for 24 h.

9. Remove the medium and add the appropriate selection medium to select stable colonies.

Note 1: Aseptic technique should be observed throughout the transfection procedure.

Note 2: For best results DNA should be free of protein and phenol. All DNA should be kept sterile after ethanol precipitation and solubilization in sterile water or Tris EDTA.