Product: Calcium Phosphate Transfection System

Cat. No.: 18306-0119  
Lot No.: HHS704  
Size: 75 Reactions

Storage Conditions: 4°C  
Do Not Freeze

COMPONENT  AMOUNT  PART NO.
10X HBS [HEPES, NaCl]  
1N NaOH  
Calcium Solution  
Phosphate Solution  
[mono- and di-basic sodium phosphate]  
Transfection Qualified Water  
Carrier DNA  
[salmon sperm DNA 10 mg/ml]  

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  
NIH3T3  
γ 2  
γ AM  
B78H1  
ATF 20  
Rat: FRTL-5  
BRL Cells  
GH3  
AR 42 J  

Monkey: COS-1  
COS-7  
CV-1  
Verro  

Human: HeLa  
C-4  
293  

Hamster: CHO

Quality Control Assays:
The Calcium Phosphate Transfection System is tested for the absence of bacterial and fungal contamination using thio glycolate 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:

Doc. Rev.: 101494
**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 $1 \times 10^6$ cells/100-mm dish/10 ml 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 ml 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.

Cat. No.:18306-019