

Calcium Phosphate Transfection Kits

Parts CP-1, CP-4

Storage:

Shipped at ambient temperature. Aliquot and store at -20°C after arrival. Thaw and use at room temperature. Reagents retain activity after multiple freezing and thawing cycles.

Important: Use sterile conditions with all liquid reagents.

Kit Contents:

- I. Calcium Phosphate Transfection Kit 1 (CP-1):** Suitable to transfect 100, 10 cm cell culture dishes.
- Sterile Calcium chloride (Cl-1; 55 mls)
 - HBS (HBS-1; 55 mls)
 - Sodium Phosphate (SP-1; 2.1 mls)

Link to product description and ordering on the Fivephoton Biochemicals website:
http://fivephoton.com/index.php?route=product/product&path=20&product_id=50

- II. Calcium Phosphate Transfection Kit 4 (CP-4):** As Kit 1, except suitable to transfect 200 10 cm cell culture dishes.
- Sterile Calcium chloride (Cl-1; 110 mls)
 - HBS (HBS-1; 110 mls)
 - Sodium Phosphate (SP-1; 4.2 mls)

Link to product description and ordering on the FIVEphoton Biochemicals website:
http://fivephoton.com/index.php?route=product/product&path=20&product_id=81

Overview: Calcium phosphate transfection reliably introduces genes into HEK293 cells and other “easily” transfected cells. The experimenter typically employs a CMV driven plasmid transfection vector, such as pCDNA3.1, with a cDNA insert of interest. Plasmid DNA (which may be precipitated) is first suspended in a calcium chloride solution, followed by the addition of the calcium chloride-DNA solution into a HEPES-Sodium Phosphate (HBS) solution, pH 7.05-7.1. The pH of the HBS solution is a stringent requirement for successful transfection: therefore, the experimenter should maintain the HBS solution at pH 7.05-7.1. Observance of a cloudy mixture following the addition of the DNA-calcium chloride solution into the HBS solution reflects that the calcium phosphate precipitation was successful and should lead to gene transfection.

Applications: Since Calcium Phosphate Transfection in HEK293 cells creates relatively high transfection yields, the technique can be readily applied to detect protein expression by Western blot, immunofluorescence, RT-PCR, radioligand receptor assays and functional assays using transient. Calcium phosphate transfection can also be used to introduce genes for stable transfection. Expression of the transfected gene can typically be observed 16-24 hrs post-transfection using transient transfection methods. However, it is generally believed that a 48 hr post-transfection period results in maximal protein expression. Transfected genes can be detected up to 72 post-transfection without G418 selection.

In addition to low toxicity, the calcium phosphate transfection method requires few handling steps, and the calcium phosphate-DNA precipitate solution can be conveniently left overnight on cells unlike the more cytotoxic liposome and cationic transfection methods. Transfection can take place in the presence of serum and antibiotics. The downside of calcium phosphate transfection is it successfully transfects a smaller subset of cell types compared to liposome transfection techniques. Calcium phosphate transfection operates effectively in HEK cells, which is the most commonly used cell culture-transfection model.

All solutions in the Fivephoton Biochemicals Calcium Phosphate Transfection Kits are provided sterile and adjusted to pH 7.1 prior to shipping. However, due to potential changes during shipping, the pH of the HBS solution should be checked, and readjusted, if needed, to pH 7.05-7.1 prior to first use with 0.1N NaOH or 0.1N HCl. No adjustment is needed with the calcium chloride and sodium phosphate solutions.

To check the pH of the HBS solution, under sterile conditions, remove 100 μl of HBS solution and dispense into 5 ml dH_2O , mix and measure the pH with a pH meter. The pH should be 7.05-7.1, which is a stringent requirement for successful transfection. If a different pH reading is observed, use 0.1N NaOH or 0.1N HCl to slowly adjust the original HBS solution to pH 7, and then sterile filter the solution. The provided plastic bottles can be autoclaved if you wish to reuse them to store the transfection reagents.

If the HBS solution is stored at room temperature, a pH check (and adjustment) is recommended approximately every two weeks. Aliquoting and storage of the HBS solution at -20°C can be expected to maintain the pH of the HBS solution during long term storage. The calcium chloride and sodium phosphate solutions do not require checking or readjustment as mentioned above.

Quick Protocol: Calcium Phosphate Transfection Kit

Quick Protocol: The protocol below is for one 10 cm cell culture dish with 10 ml of culture medium. For other sized cell culture vessels, or for simultaneous transfection of several cell culture dishes, adjust DNA amounts and transfection solution volumes to corresponding ratios of total cell culture media volume, taking into account all dishes that will be transfected. Antibiotics and serum can be present in the cell culture media at all steps during the transfection.

Reagent volumes required to transfect one 10 cm cell culture dish

- 500 µl of CALCIUM CHLORIDE (CI-1) solution,
- 500 µl HBS (HBS-1) solution,
- 20 µl SODIUM PHOSPHATE (SP-1) solution
- 20 µg of plasmid DNA to transfect.

Important: Cells should be approximately 70% confluent on the day of transfection. Sterile conditions should be maintained in all procedures.

Procedure:

1. Check, and if necessary, adjust the pH of the HBS solution (HBS-1) to pH 7.05-7.1 as described above on page 2 on first use, and once every two week period if the HBS solution is stored at RT. Perform syringe sterilization with a 2 µM filter after pH adjustment. Storage of the HBS solution at -20°C should result in stable pH maintenance, and not require further adjustment.
2. In a 1.5 ml microcentrifuge tube, precipitate 20 µg plasmid DNA for every 10 cm dish that will be transfected. Use a sodium acetate-ethanol method to precipitate DNA. If co-transfecting more than one plasmid, the total plasmid DNA mass should not exceed 20 µg for each 10 cm cell culture dish. During DNA precipitation, shake the tube to cover ethanol on all walls of the tube to sterilize the tube. Sediment the DNA using centrifugation (in a standard microcentrifuge, using maximum speed for 15 min). Next, under sterile conditions in a cell culture hood, aspirate off the ethanol, leaving the DNA pellet intact at the bottom of the tube.
3. Under sterile conditions in a cell culture hood, suspend the DNA pellet in the provided CALCIUM CHLORIDE solution (CI-1) as follows: Add 500 µl CALCIUM CHLORIDE (CI-1) solution to the DNA pellet. Flick the tube to solubilize the DNA. Carefully observe whether the DNA has been suspended. This solution is referred to as the CALCIUM CHLORIDE-DNA solution.
4. In a separate tube under sterile conditions, prepare the HBS-SODIUM PHOSPHATE solution by adding 20 µl SODIUM PHOSPHATE (SP-1) to 500 µl HBS (HBS-1) solution. Vortex the tube for 10 sec. This solution is referred to as the HBS-SODIUM PHOSPHATE solution.
5. Slowly drip the CALCIUM CHLORIDE-DNA solution into the HBS-SODIUM PHOSPHATE solution. Mix the resulting solution either by using a vortexer, or by bubbling with a mechanical-seriological pipette during the addition of the CALCIUM CHLORIDE-DNA solution into the HBS solution. The total transfection solution volume should now be 1020 µl. Let the tube stand at room temperature for 30 min to allow for precipitation. The solution should appear cloudy within the 30 min precipitation period.
6. **Transfection:** Dispense the transfection mixture drop-wise and evenly over the cell culture dish. Gently rotate the cell culture dish while dripping in the transfection solution. Place the cell culture dish back into the cell culture incubator.
7. After a 6 hr to overnight incubation of cells with the transfection solution, gently remove the cell culture media, gently rinse the cell culture dish twice with PBS and then add fresh cell culture media. Antibiotics and serum can be present at all stages of the transfection.

Detailed Protocol: Calcium Phosphate Transfection Kit

Detailed Protocol: (This protocol describes volumes of transfection reagents and DNA amounts for one 10 cm cell culture dish with 10 ml of culture medium. For other sized cell culture vessels, or for multiple culture vessels, adjust DNA amounts and transfection solution volumes to ratios corresponding to the volume of cell culture media. For example, if the cell culture medium in the dish is 1 ml, use 1/10 the amount of plasmid DNA and 1/10 the amount of each transfection reagent. Alternatively, to transfect ten 10 cm cell culture dishes, use 10X the plasmid DNA mass and 10X the amount of each transfection reagent).

Important: Sterile conditions should be maintained in all procedures. Cells should be approximately 70% confluent on the day of transfection. Transfection can take place in the presence of serum and antibiotics in the cell culture medium.

1. pH adjustment of HBS-1 (to pH 7.05-7.1) and syringe filter sterilization prior to first use: Thaw solutions to room temperature. Although the HBS solution is provided at pH 7.1 and sterilized, due to potential changes in pH during shipping, check and adjust the pH of the HBS-1 solution to 7.05-7.1 with 0.1N NaOH or 0.1N HCl. Sterilize the solution with a syringe filter and dispense the solution into a sterile tube. The sterilized HBS-1 solution can be aliquoted into smaller sterile screw-cap tubes and stored at -20°C to maintain pH.
2. DNA precipitation: For each 10 cm dish (10 ml culture medium), use an ammonium acetate or sodium acetate - 70% ethanol method to precipitate 20 µg of plasmid DNA. If co-transfecting more than one plasmid DNA in the 10 cm cell culture dish, the total amount of plasmid DNAs should also equal 20 µg. Do not use less than 20 µg DNA per 10 cm cell culture plate. DNA precipitation can be performed in a 1.5 ml snap-cap microcentrifuge tube. After adding the ethanol for DNA precipitation, close the cap and shake the tube to disperse ethanol over the walls and top of the tube to sterilize it. Observe the fibrous DNA precipitate. Centrifuge at maximum speed in a microcentrifuge at RT (15000 rpm, 15 min, RT). Place the tube in a sterile culture hood and carefully remove all ethanol by aspiration, retaining the DNA pellet at the bottom of the tube. Make sure all ethanol is removed by aspiration, taking care to maintain the DNA pellet in the bottom of the tube. This and all subsequent procedures should be performed under sterile conditions in a cell culture hood.
3. Suspend the DNA pellet in the CALCIUM CHLORIDE (Cl-1) solution. Add 500 µl CALCIUM CHLORIDE solution to the DNA pellet. Flick the tube several times to solubilize the DNA.
4. Using a separate sterile tube, prepare the HBS-SODIUM PHOSPHATE solution by adding 20 µl SODIUM PHOSPHATE (SP-1) to 500 µl HBS (HBS-1) solution. Flick the tube to mix, or vortex for about 10 sec.
5. Drip the CALCIUM CHLORIDE-DNA solution into the HBS-SODIUM PHOSPHATE solution. During this process, you may use a sterile serological pipette and a mechanical pipettor (wiped with ethanol for sterilization) to generate bubbles to mix the solutions. (To mix the solution, place the pipette in the HBS solution and generate bubbles while dripping in the DNA-calcium chloride solution). Alternatively, briefly vortex to mix the calcium chloride-DNA-HBS solution. You should observe a cloudy mixture at this step, indicating successful precipitation. Leave this solution at room temperature for 30 min to allow calcium phosphate precipitation to proceed.
6. Transfection: Slowly dispense the transfection solution (total volume 1020 µl for one 10 cm cell culture dish) drop-wise, while gently swirling the cell culture dish (antibiotics and serum can be included in the medium). After dispensing all transfection solution, place the cell culture dish in the cell culture incubator.

After six hours, to overnight incubation, observe the plate of cells under a cell culture microscope. You should observe dark precipitates on the cells. Gently remove the cell culture media under sterile conditions, gently rinse the cell culture dish 2X in sterile PBS and then add fresh cell culture media. A 16-24 hr post-transfection period may be a sufficient transfection time to observe protein expression using techniques such as Western blotting, immunoprecipitation and immunofluorescence. Alternatively, grow cells for approximately another 24 hrs prior to experimentation.

Adjusting transfection reagent volumes for other sized cell culture vessels: Use corresponding DNA masses and volumes of transfection reagents for other sized vessels based on the above protocol. For example, when transfecting a well in a 6-well culture dish that contains 2 mls of cell culture media, the amount of total plasmid DNA used in the transfection should be calculated as: $2/10 \times 20 = 4 \mu\text{g DNA}$; volume of calcium chloride solution is $2/10 \times 500 = 100 \mu\text{l}$; volume of HBS is $2/10 \times 500 = 100 \mu\text{l}$; volume of sodium phosphate is $2/10 \times 20 = 4 \mu\text{l}$.

Summary of Transfection Volumes for a 10 cm cell culture dish with 10 ml culture medium.

CALCIUM CHLORIDE	DNA	HBS	SODIUM PHOSPHATE
500 μ l	20 μ g	500 μ l	20 μ l

Troubleshooting:

1. Transfection was working consistently at high efficiency, but either stopped working or efficiency diminished. Resolution: Adjust the pH of the HBS solution to 7.05-7.1. Also use a new passage of cells.
2. Low initial transfection efficiency was observed (<10% per cent of cells were transfected). Resolution: Verify the plasmid DNA mass with a spectrophotometer. Additionally, cells should be elongated and attached; use a new passage of cells.
3. Combined calcium chloride-HBS transfection solution is not cloudy. Resolution: Adjust the pH of the HBS solution to pH 7.05-7.1. Make sure there is mixing by bubbling or vortexing during the addition of the Calcium phosphate-DNA solution into the HBS solution.
4. Solutions became contaminated with microbes. Resolution: Use a 2 μ m diameter pore size syringe filter to re-sterilize all transfection solutions. Make sure all tubes are sterile. When precipitating DNA, shake the tube to cover all walls of the tube with ethanol.