Calcium Phosphate Transfections in Corning® CellSTACK® Culture Chambers

Protocol

Below is a generalized procedure for calcium phosphate based transfection in Corning CellSTACK Chambers. It is intended as a starting point for individuals wishing to transfect large numbers of cells in the Corning CellSTACK chamber and can be adapted for each size. The procedure was developed using HEK 293 cells on Corning CellSTACK-10 chambers (which is described below). The same procedure was also performed on 2 layer chambers with similar transfection frequencies. Modifications can be made on a per layer basis for other chamber sizes.

Helpful Hints

- Prepare the $CaCl_2$ and 2X BES-buffered saline (BBS) solutions (see page 2) several days before use and freeze. Test the solutions on a small scale, in six well dishes, optimizing the time for precipitate formation prior to addition to cells.
- Positive control: use cells plated at 40,000 cells/cm^2 in a six well dish. Add 0.2 mL of the remaining large scale precipitate to each well.
- A second control should also be included with a precipitate that was formed for a standard small scale transfection.

Cell Plating

Sixteen to twenty-four hours before transfection, plate cells at a density of 40,000 cells/cm^2. Incubate at 37°C in 5% CO_2.

Four to six hours prior to transfection, change
the medium in the CellSTACK chamber to a final volume of 100 mL per layer (~0.15 mL/cm^2). Use normal growth medium without antibiotics. Place the CellSTACK chamber back in the incubator.

Precipitate Formation

1. Thaw all solutions and bring them to room temperature.
2. In a sterile 500 mL Erlenmeyer flask, add
   0.25 M $CaCl_2$ and DNA (3.18 mg) to a final volume of 31.8 mL.

   a. 0.25 M $CaCl_2$ = 0.005 mL/cm^2

   b. DNA = 0.4 to 0.6 µg/cm^2

   Note: It is important that the volume of DNA added be kept to a minimum (< 3 mL) to not dilute out the $CaCl_2$. Concentration of DNA stock is recommended to be > 2 mg/mL.
3. Mix well.
4. While mixing the $CaCl_2$/DNA mix, slowly add 31.8 mL of 2X BBS by drop wise addition. The mixture should become slightly cloudy at this time.

   a. 2X BBS = 0.005 mL/cm^2.

   Note: It is extremely important that the addition of 2X BBS buffer happen rapidly to ensure the proper formation of precipitate. Ideally the buffer should be added within 90 seconds. For most applications the precipitate can be added immediately to the CellSTACK chamber; however, short incubations (i.e., 2 to 10 minutes) prior to the addition of precipitate may also be conducted. Ultimately, the length of incubation, if necessary, is user defined and should be optimized on a small scale (i.e., in a 6 well plate) prior to transfection in the CellSTACK chamber.
5. The precipitate is now ready for addition to the CellSTACK chamber.

Addition of Precipitate to CellSTACK Chambers

1. Remove CellSTACK chamber from incubator and pool medium into lowest layers. This is done by tilting the CellSTACK chamber on a port corner to allow as much medium as possible to pool into the lowest layers (Figure 1).

   Note: This is done to facilitate mixing of the precipitate within the CellSTACK chamber.
2. Using a 50 mL pipette, pipette all precipitate prepared above to the lowest layer of the CellSTACK chamber.
3. Follow the procedure for medium equilibration in the CellSTACK chamber insert manual to equilibrate medium throughout the layers of the unit.
4. Repeat steps 1 and 3 twice more.
5. Incubate at 37°C, 5% CO_2.
6. Sixteen to 24 hours after the addition of the precipitate, perform a medium change (with antibiotics, if desired) and culture cells as necessary for transient or stable transfection protocols.

Solutions

0.25 M $CaCl_2$

- 7.35 g $CaCl_2 \cdot 2H_2O$
- 190 mL ddH_2O
- Dissolve $CaCl_2$ and bring the final volume to 200 mL with dd H_2O
- Sterile filter the solution

2X BBS

- 2.14 g N,N-bis[2-hydroxyethyl] 2-aminoethanesulfonic acid (BES)
- 3.2 g NaCl
- 0.054 g NaH_2PO_4
- Add 180 mL ddH_2O and bring the pH of the solution to pH 6.96.
- Bring the final volume to 200 mL with ddH_2O.
- Sterile filter the solution.

For typical results, see *A Cost Effective, Scalable Method for Transfection in Corning® CellSTACK® Culture Chambers*, Corning publication number CLS-AN-063.

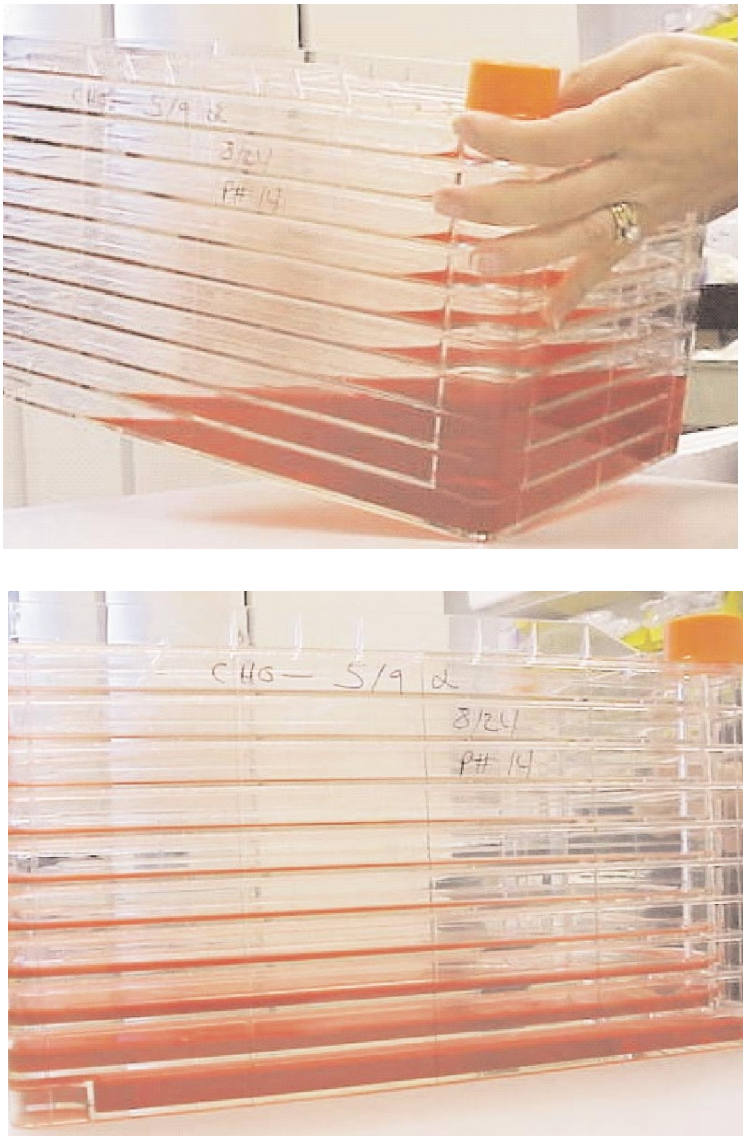

Figure 1. Top: tilting of CellSTACK® chamber to pool media in lowest layers. Bottom: Media levels after pooling into bottom layers.

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