

Corning® HYPERFlask™ Cell Culture Vessel

Calcium Phosphate Transfection Protocol



Corning HYPERFlask Cell Culture Vessel

Introduction

One of the most useful tools in cell biology research is introduction of foreign materials (such as nucleic acid) into eukaryotic cells. Transfection of mammalian cell lines with DNA is one of the most popular techniques to achieve this. The traditional strategy for achieving this is via a calcium phosphate based co-precipitation with nucleic acid. Further, in much of today's research there is a growing need to transfect large quantities of cells. The use of calcium phosphate is ideally suited for this due to its relatively good efficiency, scalability and, most importantly, its low cost. The utility of the Corning HYPERFlask vessel includes its performance in routine transfection procedures. To this end we have developed a robust protocol that can be applied and modified if necessary for your needs. The protocol was developed using Chinese hamster ovary (CHO) cells but has been successfully applied to a variety of other cell types including HeLa cells. The protocol is intended as a starting point to optimize your work. We used a Calcium Phosphate Transfection Kit (Invitrogen Cat. No. K2780-01) in developing this protocol.

Day 1

The procedure below is for plating cells into a HYPERFlask vessel and multiple wells of a 24-well plate. The 24-well plate will serve as a control for overall transfection efficiency as well as transfection efficiency of the large scale precipitate made for the HYPERFlask vessel. Should you choose to use a different size control well scale your changes in reagents based on an equivalent mL/cm².

Plating Cells

NOTE: Use early passage cultures (5 to 20 passages) at 80 to 90% confluence.

1. Seed cells at ~20,000 cells/cm² in 0.326 mL/cm² of growth media (10% FBS IMDM).

HELPFUL HINT: We recommend setting up control plates such as multiple wells of a 24-well plate or similar to track transfection efficiency (Table 1) This will include mock transfection, positive control transfection, as well as large scale precipitate made for the HYPERFlask vessel added to a well.

HELPFUL HINT: Cultures should be at 80% confluence 24 hours after plating.



Figure 1. Pour carefully to avoid foaming and bubbles. Notice tilt of flask to achieve low foaming.

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2. Incubate overnight at 5% CO₂, 37°C.

Table 1.

Culture Device	Surface Area (cm ²)	Working Medium Volume (mL)	Recommended Cell Concentration
HYPERFlask™ vessel	1720/flask	560/flask	34.4 x 10 ⁶ /flask
24 well plate (9 wells)	2/well	0.650/well	4.0 x 10 ⁴ /well

Day 2

Preparation of DNA/salt precipitate

NOTE: All work should be done in a biohood under sterile conditions.

NOTE: These steps have been modified from Invitrogen's Calcium Phosphate Transfection Protocol.

1. 3 to 4 hours prior to transfection change medium of all cultures to be transfected.

NOTE: For handling of the HYPERFlask vessel refer to the HYPERFlask Cell Culture Vessel – Instructions for Use.

HELPFUL HINT: Use a vacuum pump to gently aspirate medium out of the HYPERFlask to minimize cell loss and to reduce chance of contamination.

2. In sterile tubes (microcentrifuge or conical tubes) prepare the DNA mix for transfection (Table 2), see also helpful hint of step 1 in plating cells for transfections to be run.

Table 2.

Solution A	Per well of 24 well (0.650 mL/w)	Per HYPERFlask vessel (560 mL/flask)
2 mM CaCl ₂	2.34 µL	2.02 mL
DNA	1 µg	1 mg
H ₂ O	17.16 µL	14.8 mL
Final Volume (A)	19.5 µL	16.8 mL

3. In separate sterile tubes that can hold the combined volumes of Solution A and Solution B (i.e., 33.6 mL/HYPERFlask vessel), add the appropriate amount of Solution B (2x HBSS solution) (Table 3).

Table 3.

Solution B (2x Hepes Buffered Saline Solution - HBSS)	Per 24 well (0.650 mL/w)	Per HYPERFlask vessel (560 mL/flask)
2X HBSS	19.5 µL	16.8 mL

4. Slowly, by drop wise addition and while gently vortexing/mixing, add Solution A to Solution B.

NOTE: Solution A MUST be added to Solution B and not vice versa.

5. Incubate at room temperature for up to 30 minutes. A fine precipitate should be visible.

NOTE: Do not go longer than 30 minutes, as this will lead to precipitate that is too large for efficient transfection. Incubation time may be optimized with a shortened time if desired.



Figure 2. Any liquid inside the flask will distribute evenly into each layer of the flask when it is placed on its side.

Addition of Precipitate

1. Control 24-well plate precipitate addition:
 - a. Remove 39 μ L of media from each well to be transfected.
 - b. Slowly, drop wise add 39 μ L of DNA/salt mixture into well.
 - c. Swirl plate to mix well.
 - d. Take 39 μ L of HYPERFlask™ precipitate and add to a well of 24-well plate.

NOTE: If desired up to 3 wells can be tested for performance of the large scale precipitate in 24-well reactions without interfering with the efficiency of transfection of the HYPERFlask vessel.
 - e. Incubate in a humidified incubator at 5% CO₂, 37°C.
2. HYPERFlask vessel precipitate addition:
 - a. Gently pour out all medium from the HYPERFlask vessel into a 500 mL storage bottle or Erlenmeyer flask.
 - b. Remove 33.6 mL of medium from storage container; save this in a 50 mL sterile tube.
 - c. Slowly, by drop wise addition and while mixing, add the remainder of the large scale DNA/salt mixture into the 500 mL bottle containing medium from the HYPERFlask vessel.

HELPFUL HINT: Gently swirl the storage bottle or flask containing the medium with one hand while pipeting in the DNA/salt mix.
 - d. Gently pour medium transfection mix back into the HYPERFlask vessel.

HELPFUL HINT: Pour medium slowly down the angled neck of the vessel to prevent bubbling, see HYPERFlask Vessel – Instructions for Use.
 - e. If needed, use extra medium in 50 mL tube to bring liquid volume in the HYPERFlask vessel to the first cap thread.
 - f. Incubate in a humidified incubator at 5% CO₂, 37°C.
3. Process transfected cells as necessary.

Please visit the Corning Life Sciences web site to view a video presentation that describes the proper handling of the HYPERFlask™ Cell Culture Vessel.

For additional product or technical information, please e-mail us at CLStechserv@corning.com, visit our web site www.corning.com/lifesciences or call 1.800.492.1110. Outside the United States call 978.442.2200.

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