

Corning® HYPERFlask® Cell Culture Vessel jetPEI™ Transfection Protocol

Protocol

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Introduction

One of the most useful tools in cell biology research is transfection, the introduction of foreign DNA into eukaryotic cells. In much of today's research, there is a growing need for the effective transfection of large quantities of cells. Polyplus-transfection's jetPEI transfection reagent is a highly efficient, low toxicity, water-soluble polymer that can be used in the presence of serum in culture media. Therefore, there is no need to change the culture medium before or after transfecting cells, making this method ideally suited for use with the Corning High Yielding Performance Flask (HYPERFlask) cell culture vessel. This protocol was optimized using HeLa cells but has been successfully applied to a variety of cell types including Chinese hamster ovary (CHO) cells. This protocol is intended as a starting point that can be optimized by the end user for their cell lines.



Day 1

This procedure describes plating cells into a HYPERFlask cell culture 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 cell culture vessel. Once the procedure has been optimized for the HYPERFlask vessel, these samples are unnecessary. Should you choose to use a different size control well, scale your changes in reagents based on an equivalent mL/cm².

Read the protocol completely before starting procedure.

Plating Cells

Helpful Hint: For handling of the HYPERFlask cell culture vessel refer to the HYPERFlask Cell Culture Vessel Instructions for Use Manual.

Note: For best results, use early passage cultures (5 to 20 passages) at 80 to 90% confluence.

1. Seed cells at 20,000 cell/cm² in 0.326 mL/cm² of growth media (6.13 e⁴ cell/mL) into HYPERFlask cell culture vessel (Cat. No. 10010) and 24 well plate (Cat. No. 3524), see Table 1. These cell numbers should be optimized for your cell line. Cultures should be at 80% confluence 24 hours after plating.

Table 1. Medium and Cell Requirement

| | Growth Area (cm ²) | Media Vol. (mL) | Cell Concentration |
|------------|--------------------------------|-----------------|-------------------------------|
| HYPERFlask | 1720/flask | 560 mL/flask | 34.4 x 10 ⁴ /flask |
| 24 well | 2/well | 0.650 mL/well | 4.0 x 10 ⁴ /well |

Helpful Hint: We recommend setting up control wells on a 24 well plate or similar to track transfection efficiency. Controls should include mock transfection, positive control transfection, as well as controls for the large scale precipitate made for the HYPERFlask cell culture vessel.

2. Incubate overnight in 37°C humidified incubator at 5% CO₂.

Day 2

Preparation of jetPEI™/DNA complex

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

Steps have been modified from Polyplus' jetPEI transfection protocol using jetPEI transfection kit (Part. No. 101-40N) optimized for 1 µg DNA and a jetPEI N:P ratio of 5.

1. DNA solution – Solution A*

| Solution A | For One 24 Well Mock (0.650 mL/Well) | For One 24 Well Control (0.650 mL/Well) | For One HYPERFlask® (560 mL/Flask) |
|---------------------|---|--|---------------------------------------|
| DNA | – | 0.5 µg/cm ² (1 µg) | 0.5 µg/cm ² (860 µg) |
| 150 mM NaCl | 50 µL | To 50 µL | To 43.12 mL |
| Final Volume | 50 µL | 50 µL | 43.1 mL |

*Prepare in a container/tube that can hold 2x the final volume.

2. JetPEI Solution B**

| Solution B | For One 24 Well Mock (0.650 mL/Well) | For One 24 Well Control (0.650 mL/Well) | For One HYPERFlask (560 mL/Flask) |
|---------------------|---|--|--------------------------------------|
| JetPEI Reagent | 2 µL | 2 µL | 1.72 mL |
| 150 mM NaCl | 48 µL | 48 µL | 41.4 mL |
| Final Volume | 50 µL | 50 µL | 43.1 mL |

**Optimized for an N:P ratio of 5.

3. Rapidly, add the jetPEI solution B into DNA solution A, and mix well by vortexing.

Important Note: Do not add in reverse order.

Note: All mock or control replicates can be made as one cocktail and split over each well.

| Final Volume | For One 24 Well | For One HYPERFlask |
|--------------------|-----------------|--------------------|
| JetPEI/DNA Complex | 100 µL | 86.2 mL |

4. Incubate at room temperature for 30 min. Solution may appear cloudy.

Transfection

1. HYPERFlask cell culture vessel

1.1. Gently pour all medium from the HYPERFlask cell culture vessel into a sterile 500 mL storage bottle or Erlenmeyer flask. Aspirate all medium from 24 well HYPERFlask control wells.

1.2. Remove 86.2 mL of medium from 500 mL storage container, save 40 mL in a 50 mL tube.

1.3. Slowly, while mixing, add 86.2 mL of jetPEI/DNA complex into a 500 mL bottle containing medium from the HYPERFlask vessel.

1.4. Gently pour medium/precipitate mix back into the HYPERFlask cell culture vessel.

Helpful Hint: If needed, use extra medium in 50 mL tube to bring liquid volume in the HYPERFlask cell culture vessel to the first thread.

1.5. Recap and gently tap to collect all air bubbles in the air trap.

1.6. Remove 0.650 mL/well of media from HYPERFlask cell culture vessel and add in triplicate to 24 well HYPERFlask control wells.

Note: Up to 3 wells can be tested for performance of the large scale HYPERFlask vessel complex (Step 1.3) in 24 well plate without interfering with the efficiency of transfection of the HYPERFlask cell culture vessel.

2. Mock and control 24 well plate

2.1. Remove 100 μ L of medium from all control and mock wells.

2.2 Slowly, drop wise add 100 μ L of DNA/jetPEI™ complex or mock solution into corresponding wells. Swirl plate around to mix well.

| Final Volume | One 24 Well | HYPERFlask® |
|---------------------|--------------------|--------------------|
| mL | 0.650 | 560 |
| mL/cm ² | 0.326 | 0.326 |

3. Return vessel and 24 well plate to humidified 37°C incubator at 5% CO₂ and incubate for 48 hours.

4. 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 www.corning.com/lifesciences, or call 1.800.492.1110. Outside the United States, please call 1.978.442.2200.

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