Corning® HYPER*Flask*® 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. The jetPEI transfection reagent (Polyplus-transfection™) 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 **H**igh **Y**ield **PER**formance Flask (HYPER*Flask*) 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 HYPER*Flask* 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 HYPER*Flask* vessel. Once the procedure has been optimized for the HYPER*Flask* 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/cm^2.

Read the protocol completely before starting procedure.

Figure 1. Venting position to be used when pre-warming the HYPER*Flask* and HYPER*Flask* M vessels when the vessel is empty or contains low volumes of liquid for prolonged periods of time (greater than 60 minutes).

Helpful Hint: If choosing to pre-warm the vessel empty prior to seeding or when using low volumes during protocols such as trypsinization, transfections, etc. for prolonged periods of time (greater than 60 minutes), vent the vessel with the cap in the venting position as shown in Figure 1. This will allow proper ventilation to prevent pressure build-up. *Please note*: This includes storing the empty HYPER*Flask* vessels for periods of time greater than 60 minutes in the incubators of The Automation Partnership (TAP) SelecT™ and CompacT SelecT™ automated cell culture systems.

Plating Cells

Helpful Hint: For handling of the HYPER*Flask* vessel refer to the HYPER*Flask* M Cell Culture Vessel Instructions for Use, CLS-CC-029.

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

1. Seed cells at 20,000 cell/cm^2 in 0.326 mL/cm^2 of growth media (6.13 e^4 cell/mL) into the HYPER*Flask* cell culture vessel (Corning Cat. No. 10024) and 24 well plate (Corning 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	Media Volume	Cell Concentration
HYPER*Flask* vessel	1720 cm^2/flask	560 mL/flask	34.4 x 10^6/flask
24 well plate	2 cm^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 HYPER*Flask*® vessel.

2. Incubate overnight in a 37°C humidified incubator at 5% CO_2.

Day 2

Preparation of jetPEI™/DNA complex

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

Steps have been modified from the jetPEI™ transfection protocol using the jetPEI transfection kit (Polyplus-transfection™ Cat. 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 Plate (0.650 mL/Well)	For One 24 Well Control Plate (0.650 mL/Well)	For One HYPER*Flask* Vessel (560 mL/Flask)
DNA	–	0.5 µg/cm² (1 µg)	0.5 µg/cm² (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 A	For One 24 Well Mock Plate (0.650 mL/Well)	For One 24 Well Control Plate (0.650 mL/Well)	For One HYPER*Flask* Vessel (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 Plate	For One HYPER*Flask* Vessel
jetPEI/DNA Complex	100 µL	86.2 mL

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

Transfection

1. HYPER*Flask* cell culture vessel

   1.1. Gently pour all medium from the HYPER*Flask* vessel into a sterile 500 mL storage bottle or Erlenmeyer flask. Aspirate all medium from 24 well HYPER*Flask* 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. While mixing, slowly add 86.2 mL of jetPEI/DNA complex into a 500 mL bottle containing medium from the HYPER*Flask* vessel.

   1.4. Gently pour medium/precipitate mix back into the HYPER*Flask* vessel.

   Helpful Hint: If needed, use extra medium in 50 mL tube to bring liquid volume in the HYPER*Flask* vessel to the first thread.

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

Helpful Hint: Due to the direct contact of the vessel cap with culture medium, it is recommended to change the cap when culturing for prolonged periods of time or when opening and closing the vessel repeatedly. This will help to reduce the possibility of contamination and ensure that a sufficient seal is obtained. For your convenience, additional caps are available (Cat. No. 10035).

1.6. Remove 0.650 mL/well of media from HYPER*Flask*® vessel and add in triplicate to 24 well HYPER*Flask* control wells.

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

2. 24 well mock and control plates

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

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

Final Volume	**One 24 Well**	**HYPER*Flask* Vessel**
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% CO_2 and incubate for 48 hours.

4. Process transfected cells as necessary.

Please visit the Corning Life Sciences website to view a video presentation that describes the proper handling of the HYPER*Flask* 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.

CORNING

Corning Incorporated
Life Sciences
Tower 2, 4th Floor
900 Chelmsford St.
Lowell, MA 01851
t 800.492.1110
t 978.442.2200
f 978.442.2476

www.corning.com/lifesciences

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All Other European Countries
t 31 (0) 20 659 60 51
f 31 (0) 20 659 76 73

LATIN AMERICA

Brasil
t (55-11) 3089-7419
f (55-11) 3167-0700

Mexico
t (52-81) 8158-8400
f (52-81) 8313-8589

Corning Incorporated, One Riverfront Plaza, Corning, NY 14831–0001