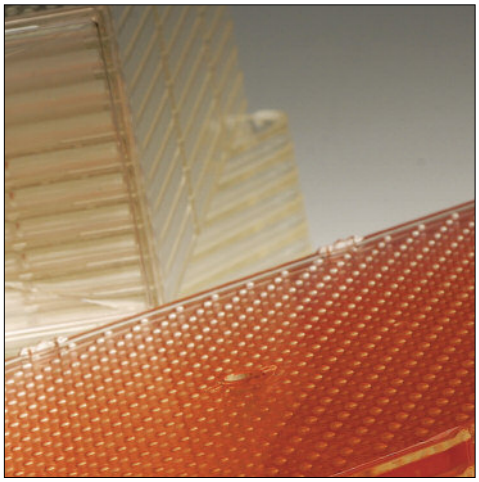
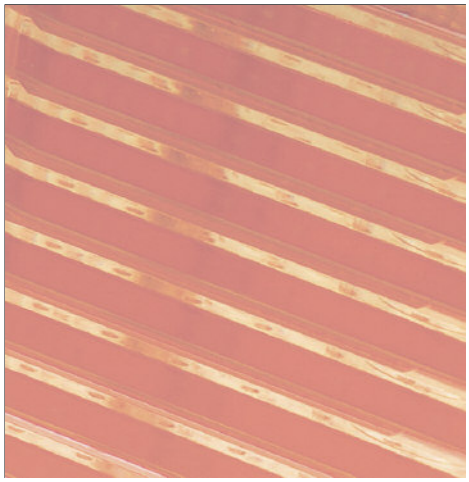
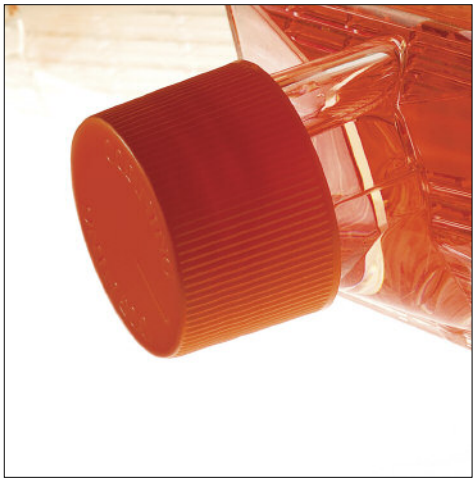
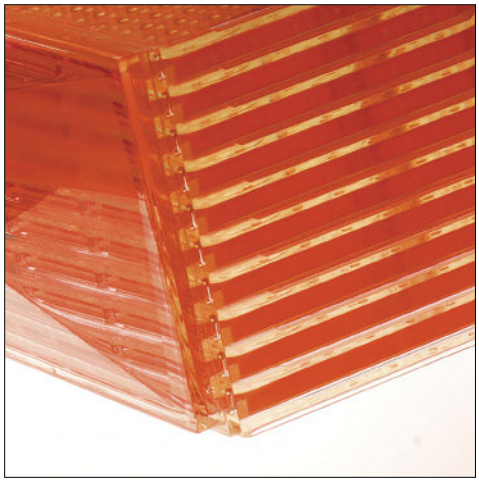
Corning® HYPER*Flask*® Cell Culture Vessel jetPEI™ Fast Transfection Protocol

Protocol

This is an optimized rapid version of the standard jetPEI High Yielding Performance *Flask* (HYPER*Flask*) cell culture vessel transfection protocol (CLS-AN-110). It was developed to save time and cost by transfecting and seeding the HYPER*Flask* vessel in one step.

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 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.

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Cell Culture Suspension Set Up

Steps were modified from Corning HYPER*Flask*® Cell Culture Vessel, jetPEI™ Transfection Protocol and Polyplus-transfection's jetPEI™ transfection protocol.

The procedure described below is for plating cells into a HYPER*Flask* cell culture vessel (Corning Cat. No. 10010 or 10024) and multiple wells of a 24 well plate (Corning Cat. No. 3524). The 24 well plate will serve as a control for overall transfection efficiency as well as the transfection efficiency of the large scale precipitate made for the HYPER*Flask* vessel. Should you choose to use a different size control well, scale your changes in reagents based on an equivalent mL/cm^2 (Table 1).

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

1. Harvest flask using standard harvest techniques, centrifuge for 10 min at 1,000 rpm and re-suspend cells in 50 mL of fresh growth medium.

   Helpful Hint: If necessary, pass cell suspension through cell strainer to achieve a single cell suspension.

2. Prepare a cell suspension at 1.82 x 10^5 cells/mL in a fnal volume of 424 mL for each HYPER*Flask* vessel to be tested using fresh growth medium.

3. Prepare a cell suspension at 1.82 x 10^5 cells/mL in a final volume of 550 µL for each control or mock well to be tested.

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

JetPEI/DNA Complex

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 A. Prepare in a container or tube that can hold 2X the final volume.

Solution A*	**For One 24 Well Mock (0.650 mL/Well)**	**For One 24 Well Control (0.650 mL/Well)**	**For One HYPER*Flask* Vessel (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 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, while gently mixing add jetPEI solution B into DNA solution A; 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 HYPER*Flask* Vessel**
JetPEI/DNA Complex	100 µL	86.2 mL

4. Incubate at room temperature for 15 min. Solution may appear cloudy.

Transfection

For handling of the HYPER*Flask*® cell culture vessel refer to the HYPER*Flask* Cell Culture Vessel Instructions for Use.

HYPER*Flask* Cell Culture Vessel

1. Slowly, while mixing, add 86.2 mL jetPEI™/DNA complex to the cell suspension.

   Note: Minimize foaming of medium when mixing.

2. Seed 650 µL/well of cell/DNA complex solution to 24 well control plate to serve as the large scale precipitate controls.

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

3. Gently pour remaining solution into HYPER*Flask* vessel, remove all trapped air and recap securely

   Helpful Hint: If necessary, use fresh growth medium to bring liquid volume in the HYPER*Flask* vessel to the first thread.

Control Wells

4. Slowly, while gently mixing add the jetPEI/DNA complex to cell suspension.
5. Seed 650 µL/well of the correct solution into corresponding control wells.
6. Incubate all cultures at 5% CO_2, 37°C for 48 hours.
7. 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 HYPER*Flask* 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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