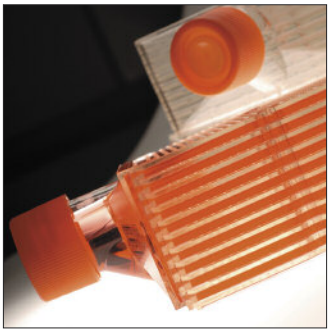
Corning® HYPER*Flask*® Cell Culture Vessel

Calcium Phosphate Transient Transfection



SnAPPShots

A brief technical report from the Corning Applications Group

Pilar Pardo
Corning Incorporated
Life Sciences
Kennebunk, ME

Introduction

The introduction of foreign deoxyribonucleic acid (DNA) into a eukaryotic cell to change or alter phenotypic expression of a gene is a commonly used tool. In transient transfections, a DNA sequence is introduced into the cells and maintained, extrachromosomally, for short periods of time ranging from 12 to 80 hours (1). In much of today's research, there is a growing need to carry out transient transfections in a large numbers of cells. The Corning High Yielding Performance Flask (HYPER*Flask*) is ideally suited to help researchers meet this goal. A diversity of methods exist for transfecting cell lines; in this study, we used the calcium phosphate ($CaPO_4$) protocol to demonstrate the scalability and performance of transient transfections using the HYPER*Flask* vessel.

Calcium phosphate transfection works by facilitating the binding of DNA to the cell membrane through the formation of a DNA/salt precipitate. The DNA then enters the cell through normal endocytic pathways. This method of transfection is commonly used due to its lower cost and relatively good efficiency with a variety of cell lines. In this application note, we transfect both HeLa and CHO-K1 cells using a plasmid that codes for a secreted form of embryonic placental alkaline phosphatase (pSEAP, Clontech®) and a plasmid that codes for the expression of a green fluorescent protein (GFP) (gWIZ GFP™, Genlantis Inc.). We also demonstrate the versatility of the vessel by comparing a standard calcium phosphate transfection method (2) to a modified fast transfection method that reduces reagent usage and time by transfecting and seeding cells simultaneously.

Method and Materials

Transfections were carried out according to *Corning* HYPER*Flask Cell Culture Vessels; Calcium Phosphate Transfection Protocol* (CLS-AN-092, available at www.corning.com/lifesciences).

For these studies, the standard transfection protocol was used to transfect cells with pSEAP DNA and the fast transfection protocol to transfect cells with gWIZ GFP DNA (Genlantis, Inc). A 24 well plate was used to monitor the overall transfection efficiency, as well as the transfection efficiency of the large-scale DNA $CaPO_4$ salt complex prepared for the HYPER*Flask* vessel transfection. Three wells per condition (mock, positive control and HYPER*Flask* vessel control) were set up for each preparation. Due to the extreme sensitivity of calcium phosphate to changes in pH (3), all work was done using 5% CO_2 and 37°C equilibrated growth medium. Iscove's modified Dulbecco's medium (IMDM) (MediaTech) supplemented with 10% FBS (MediaTech) was used as growth medium. For transfections, growth medium was allowed to equilibrate in a 5% CO_2 incubator for 24 hours using a 3L disposable spinner flask (Corning Cat. No. 3563).

Early passage cultures of exponentially growing CHO-K1 (ATCC® Cat. No. CCL-61) cells were used for the standard transfection method. Cells were plated at 20,000 cells/cm^2 in 0.326 mL/cm^2 of growth medium into a 24 well plate (Corning Cat. No. 3337) and a HYPER*Flask* vessel (Corning Cat. No. 10024) as indicated in Table 1 and incubated overnight. Four to five hours prior to transfection, a complete medium change was done using equilibrated growth media.

CORNING

For the fast transfection method, early passage cultures of exponentially growing HeLa (ATCC® Cat. No. CCL-2) cells were harvested, centrifuged for 5 minutes at 1,000 rpm and re-suspended in fresh growth medium. A cell suspension of 1.23 x 10^5 cells/mL (40,000 cells/cm^2 in 0.326 mL/cm^2) was prepared using equilibrated growth medium. The cell suspension was prepared taking into account the volume of DNA/$CaPO_4$ salt complex required to transfect the cells as shown in Table 2.

The DNA solution was prepared using 0.5 µg/cm^2 of pSEAP (Clontech®) or 0.25 µg/cm^2 of gWIZ GFP™ DNA, 2 mM $CaCl_2$ and cell-culture-grade water as indicated in Table 3. The DNA solution was added slowly, while gently mixing, to an equal volume of 2X HBS solution (Table 3) and allowed to incubate at room temperature for 10 minutes. After incubation, the DNA/$CaPO_4$ salt complex was slowly mixed with growth medium and gently added to either plated cells (standard method), or to cells in suspension (Table 3) and seeded into a HYPER*Flask*® vessel and control wells (fast method). Once transfected, cells were incubated in a humidified incubator at 5% CO_2 and 37°C for 48 hours.

Measuring Transfection Efficiency

Transfection efficiency was determined by monitoring the production of secreted embryonic placental alkaline phosphatase (SEAP) and expression of green fluorescent protein (GFP). Successfully transfected cells expressing the pSEAP plasmid will secrete placental alkaline phosphatase into the growth medium. To determine SEAP production, 110 µL of medium from each condition was collected and processed

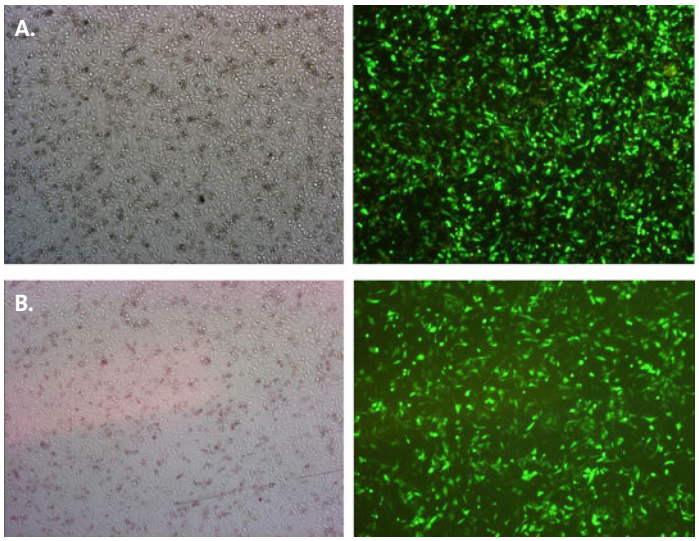


Figure 1. HeLa cells expressing GPF after successful transfection using gWIZ GFP plasmid (Genlantis, Inc). (A) Cells transfected in 24 well plate as controls. (B) Cells transfected in HYPER*Flask* vessel. Images obtained using an Olympus® IMT-2 inverted fluorescence and phase contrast tissue culture microscope. Magnification, 40X.

48 hours after transfection following Clontech's Great EscAPe™ SEAP Chemiluminescence Detection Kit protocol (Cat. No. 631701). Prepared aliquots were then transferred to Corning® 96 well solid white plates (Cat. No. 3912) and the luminescent signal read using an LJL Analyst (Molecular Devices) microplate reader. Alternatively, cells successfully transfected with the gWIZ GFP plasmid will express GFP (Fig. 1). Forty-eight hours after transfection, the cells were gently detached using trypsin, and the percent of GFP

Table 1.

	Growth Area (cm^2)	Media Volume (0.326 mL/cm^2)	Cell Concentration (20,000 cells/cm^2)	Cell Concentration (40,000 cells/cm^2)
HYPER*Flask* Vessel	1720/flask	560 mL/flask	34 x 10^6/flask	69 x 10^6/flask
24 Well Microplate	2/well	0.650 mL/well	4 x 10^4/well	8 x 10^4/well

Table 2.

	24 Well Plate Mock	24 Well Plate Control	HYPER*Flask* Vessel
Cell Suspension Volume	1.23 x 10^5 cells/mL	1.23 x 10^5 cell/mL	1.23 x 10^5 cells/mL
DNA/$CaPO_4$ Complex Volume	39 mL	39 mL	33.6 mL
Equilibrated Growth Medium	To 0.650 mL	To 0.650 mL	To 563 mL
Final Volume	**0.650 mL**	**0.650 mL**	**563 mL**

Table 3.

	24 Well Plate Mock	24 Well Plate Control	HYPER*Flask* Vessel
DNA Solution			
2 mM $CaCl_2$ (1.17 µL/cm^2)	2.34 µL	2.34 µL	2.02 mL
DNA (0.5 µg/cm^2 or 0.25 µg/cm^2)		1 mg or 0.5 mg	860 mg or 430 mg
dIH_2O	17.16 µL	16.79 µL	14.7 mL
Volume	19.5 µL	19.5 µL	16.8 mL
2X HEPES Buffered Saline (2X HBS)			
2X HBS	19.5 µL	19.5 µL	16.8 mL
Final Complex Volume	**39 µL**	**39 µL**	**33.6 mL**

expression was quantified using a Guava® EasyCyte™ Mini System (Guava Technologies). Data analysis was performed using the Express® Plus software. Cell yields and viability were also determined through Trypan blue exclusion using a BioProfile® FLEX instrument (Nova® Biomedical).

Results

SEAP production was used to measure the effectiveness of the calcium phosphate transfection. Production was converted to mg/cm^2 to compare 24 well controls and HYPER*Flask*® vessel yields. The CHO-K1 cell line showed comparable transfection, as determined by the secretion of alkaline phosphatase whether the transfection was performed in a 24 well plate (58 mg/cm^2 ± SD) or in a HYPER*Flask* vessel (59 mg/cm^2 ± SD) (Fig. 2).

Since measuring efficiency by this method indirectly measures transfection, GFP expression was used to determine the actual number of transfected cells in each vessel. As stated above, cells were plated using cells/cm^2 to allow for direct comparison between the 24 well controls and the HYPER*Flask* vessel. HeLa cell cultures transfected with gWIZ GFP™ plasmid DNA had increased GFP expression in HYPER*Flask* cell culture vessels (72% ± SD) when compared to 24 well plate controls (32% ± SD) and 24 well HYPER*Flask* controls (56 %± SD) (Fig. 3A). Trypan blue exclusion cell counts show similar yields between control wells and HYPER*Flask* vessel with acceptable cell viability (>90%) for all conditions tested (Fig. 3B).

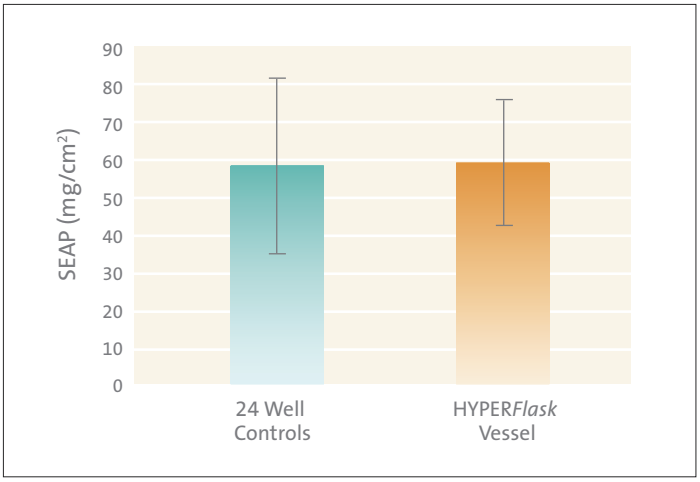


Figure 2. To measure transfection effectiveness, CHO-K1 cells were transfected with the pSEAP plasmid (Clontech®), and production of placental alkaline phosphatase was measured using Great EscAPe SEAP Chemiluminescence Detection Kit (Clontech).

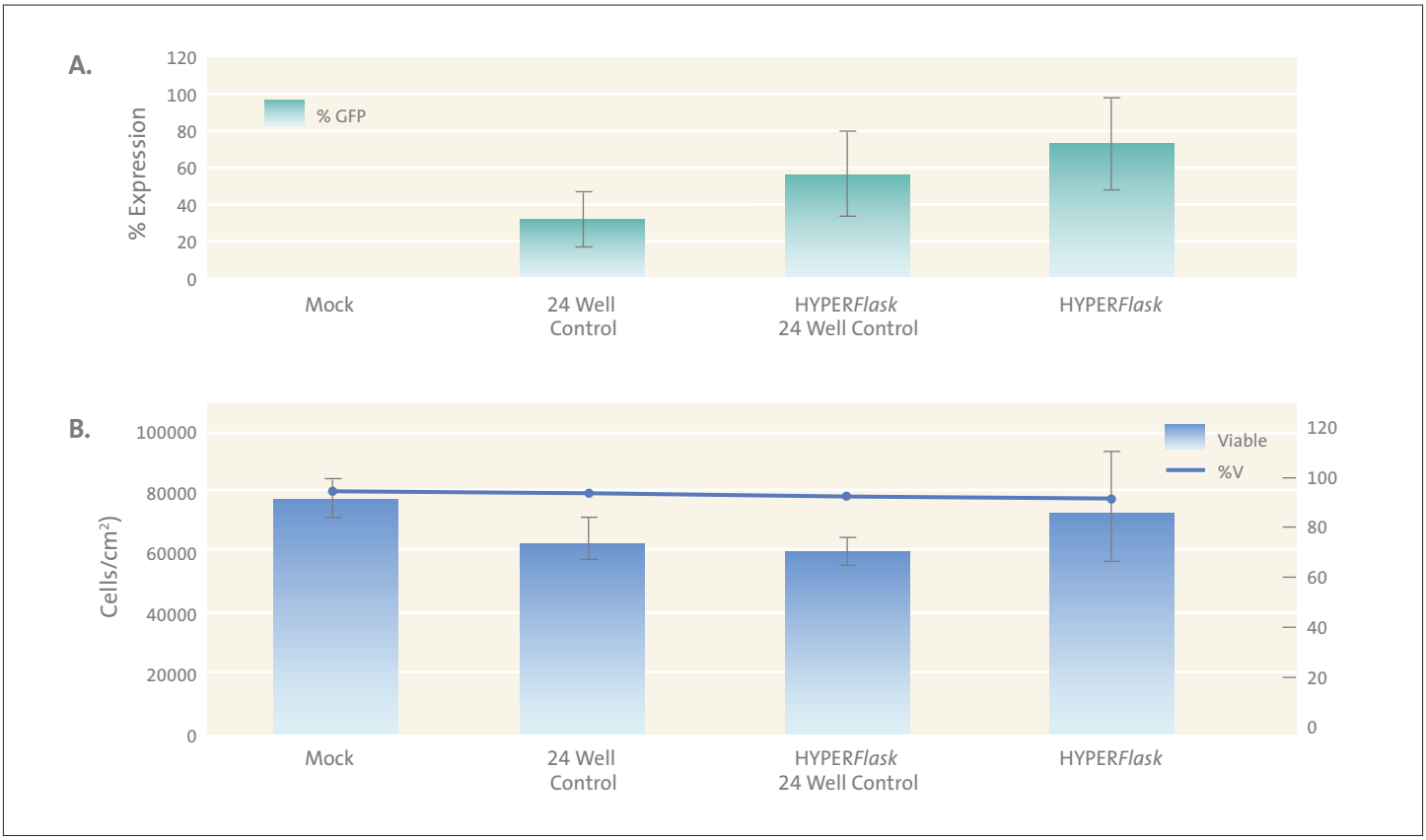


Figure 3. (A) To measure transfection efficiency, the gWIZ GFP plasmid was transfected into HeLa cells, and GFP expression was measured using a Guava EasyCyte Mini System. (B) Cell yields and viability were determined through Trypan blue exclusion using a BioProfile FLEX instrument (Nova Biomedical) (n=3).

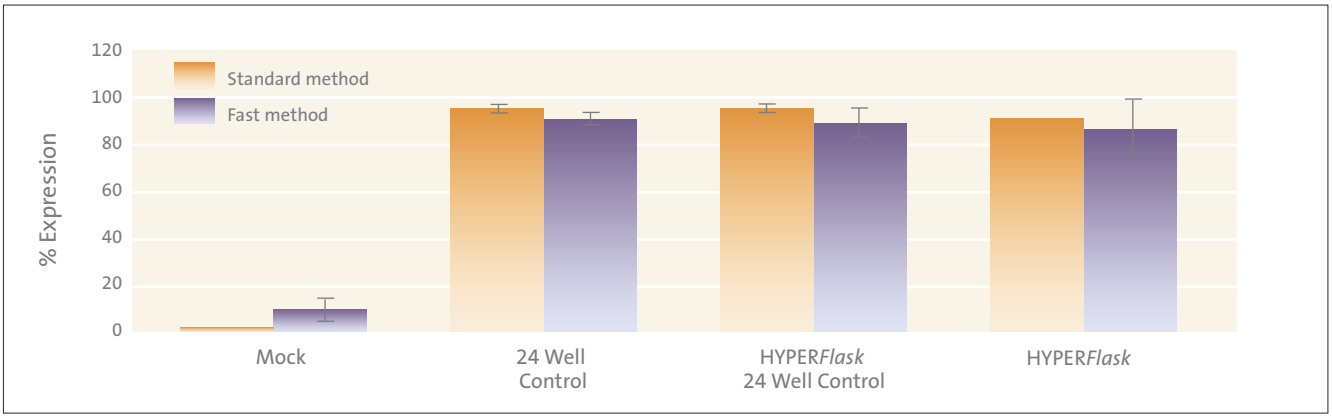


Figure 4. To compare the transfection efficiency standard vs. fast transfection method, the gWIZ GFP™ plasmid was transfected into HeLa cells and GFP expression measured using a Guava® EasyCyte™ Mini System (n=3).

A standard calcium phosphate transfection method requires preseeding cells 24 hours prior to transfection, as well as a complete media change 3 to 4 hours prior to transfecting. Both of these steps increase experimental time, reagent cost and the risk of contaminating cultures. In an effort to streamline the transfection method for the HYPER*Flask*® cell culture vessel, a fast transfection method was developed where cells are transfected in suspension and then seeded onto the vessel. With this method, experimental time was reduced by one day, reagent cost was reduced by eliminating the media change step, and the overall risk of contamination is lessened by decreasing the handling of cultures. The effectiveness of the modified fast protocol was determined by comparing the GFP expression of HeLa cell cultures transfected with gWIZ GFP plasmid DNA using both calcium phosphate tranfection methods. GFP expression showed comparable transfection between the two methods (Fig. 4).

Conclusions

- Cell transfection efficiency using the calcium phosphate protocol utilizing the Corning® HYPER*Flask* vessel was comparable to transfection levels in 24 well control wells in SEAP production, and improved in GFP expression.
- Transfection time and cost can be minimized through the use of the fast transfection protocol without compromising results or data quality.
- The Corning HYPER*Flask* cell culture vessel is a valuable option for rapid transfection of large amounts of cells.

References

1. P. Stanley. (2004) Lab Wiki: Transfections of cells with DNA, http://stanxterm.aecom.yu.edu/wiki/index.php?page=Transfection.
2. Calcium Phosphate Transfection Protocol, https://resources.invitrogen.com/content/sfs/manuals/capo4_man.pdf. Invitrogen™ Life Technologies.
3. Jordan, M and Wurm F. (2004) Methods 33:136-143.

For additional product or technical information, please visit **www.corning.com/lifesciences** or call 1.800.492.1110. Outside the United States, please call 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

Worldwide Support Offices

ASIA/PACIFIC

Australia/New Zealand
t 0402-794-347

China
t 86-21-5467-4666
f 86-21-5407-5899

India
t 91 124 4604000
f 91 124 4604099

Japan
t 81 3-3586 1996
f 81 3-3586 1291

Korea
t 82 2-796-9500
f 82 2-796-9300

Singapore
t 65 6733-6511
f 65 6861-2913

Taiwan
t 886 2-2716-0338
f 886 2-2716-0339

EUROPE

France
t 0800 916 882
f 0800 918 636

Germany
t 0800 101 1153
f 0800 101 2427

The Netherlands
t 31 20 655 79 28
f 31 20 659 76 73

United Kingdom
t 0800 376 8660
f 0800 279 1117

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