Increased Cell Yields for Cell Based Assays Using the Corning® HYPER*Flask*™ Cell Culture Vessel

SnAPPShots
A brief report from the Corning Applications Group

Hilary A. Sherman,
Ana Maria P. Pardo, and
Todd Upton, Ph.D.

Corning Incorporated
Life Sciences
Kennebunk, Maine

Introduction

The need for large quantities of cells for HTS cell-based assays continues to motivate organizations to search for methods to achieve larger cell numbers with minimal investment. The challenge is to generate large quantities of cells that all behave the same in cell based assays. In order to provide a solution it is imperative that the cells generated using such methods exhibit similar characteristics such as growth kinetics and response to stimuli. The Corning HYPER*Flask* vessel has the same overall dimensions as a standard T175 flask but was developed to generate approximately 10 times as many cells. A single HYPER*Flask* vessel can be used to seed as many as 100 384 well plates. Here we looked at the performance of two common cell lines; HEK Cre-Luc and M1WT2 cells grown on the HYPER*Flask* vessel in comparison to traditional T flasks by conducting luminescence reporter gene and calcium mobilization assays. The results show that the HYPER*Flask* vessel is capable of expanding existing cell culture capacities ~10 fold, thus increasing the capability of HTS cell-based assays without affecting cell function.

Materials and Methods

For the calcium mobilization assay M1WT2 cells (CHO-K1 cells transfected with the muscarinic receptor), cells were seeded into T175 flasks (Corning Cat. No. 431306) and HYPER*Flask* (Corning Cat. No. 10010) vessels at a concentration of 1 x 10^4 cells/cm^2 in Ham's F12 medium (Mediatech) supplemented with 10% FBS and 0.1 mg/mL G418. Cells were incubated (humidified, 37°C and 5% CO_2) for 72 hours. After 72 hours, cells were harvested with HyQTASE dissociation solution (Hyclone) and enumerated using trypan blue exclusion and a Z2 series particle counter (Beckman Coulter). Cells were centrifuged and resuspended in Ham's F12 medium supplemented with 2% FBS and 0.1 mg/mL G418 at a concentration of 3x10^5 cells/mL. One hundred microliters of each cell suspension (3x10^4 cells/well) was seeded into half of a 96 well black clear bottom tissue culture treated microplate (Corning Cat. No. 3904) and cultured for 24 hours. Using the FLIPR® Calcium 3 Assay kit (Molecular Devices, Part No. R8091) plates were processed following the manufacturers recommended protocol using carbachol to generate an intracellular calcium response. Plates were read on a Flexstation II 384 (Molecular Devices). HEK Cre-Luc cells (HEK-293 cells transfected with firefly luciferase gene under the control of a cAMP response element promoter) were seeded into a HYPER*Flask* vessel and T175 flasks at a concentration of 1x10^4 cells/cm^2 in IMDM supplemented with 0.1 mg/mL Hygromycin and 10% FBS. Flasks were incubated for 72 hours in a humidified incubator at 37°C and 5% CO_2. After 72 hours cells were harvested using HyQTASE and resuspended in IMDM without phenol red (Gibco) supplemented with 10% FBS. Cells were enumerated using trypan blue exclusion and a Z2 series particle counter and brought to a concentration of 2x10^5 cells/mL. Before seeding cells and inducing them, 5 µL of Forskolin was added to each well of a 384 well white plate (Corning Cat. No. 3570) in a serial dilution starting at a concentration of 120 µm. Twenty five microliters of cell suspension from each flask was added to half of the plate and then the plate was returned to the incubator. The next day, steadylite lyophilized substrate (PerkinElmer No. 6016989) was resuspended per vendor's protocol and 25 µL was added to each well. Plates were incubated in the dark for 5 minutes and then read on an LJL Aquest (Molecular Devices). Relative luminescence was measured and were compared for signal to background ratio, CV's and Z' assays with cells from each vessel.

Results

The calcium mobilization assay is a fluorescence based assay that works by measuring changes in intracellular calcium. M1WT2 cells are stably transfected cells that respond to carbachol stimulation by producing calcium. Carbachol dose response studies were repeated 3 times (results shown below), in order to compare the calcium response of cells grown on the HYPER*Flask* vessel as well as traditional T flasks. In this assay both flasks were found to have no significant difference in kinetics of carbachol dose response, signal to background ratio, CV's, or Z' (Figures 1 and 2). Collectively, these results indicate that cells grown in the HYPER*Flask* vessel behave identically to those grown in traditional T flasks and are suitable for use in standard cell based assay high throughput screens.

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The luciferase reporter gene assay is a luminescence based assay that measures luciferase expression. Forskolin was used to induce cAMP levels in a dose response manner on stably transfected HEK-Cre-Luc (cAMP response element-luciferase) cells. Forskolin dose response studies were repeated at least 3 times (results shown below), in order to compare the response of cells grown on the HYPER*Flask* vessel as well as traditional T flasks. Cells grown in the HYPER*Flask* vessel were shown to have lower CV's than the assays performed from traditional T flasks (Figure 3). This indicates that the cell population from the HYPER*Flask* vessel is more uniform and responds more consistently than traditional T flasks in the assay. Subsequently, this results in better Z's (Figure 3). Interestingly, this cell type grown in the HYPER*Flask* vessel did show a greater assay window than traditional T flasks as evidenced by the nearly 3 fold greater signal to background ratio (Figure 4). Importantly, the EC_{50} for both vessels is similar indicating that the HYPER*Flask* vessel provides identical or improved assay quality and results. This latter result also indicates that for some cell lines the HYPER*Flask* vessel produces cells with an improved assay window.

Conclusions

- Cells grown in the Corning® HYPER*Flask* vessel perform as good as or better than cells grown in a T175 flask in signaling assays.
- Assay performance for cells grown in the HYPER*Flask* vessel were better for both CV and Z'
- HEK Cre-Luc cells grown in the HYPER*Flask* vessel have an approximate three fold greater assay window than cells grown in T175 flasks.

Figure 1. Carbachol Dose Response

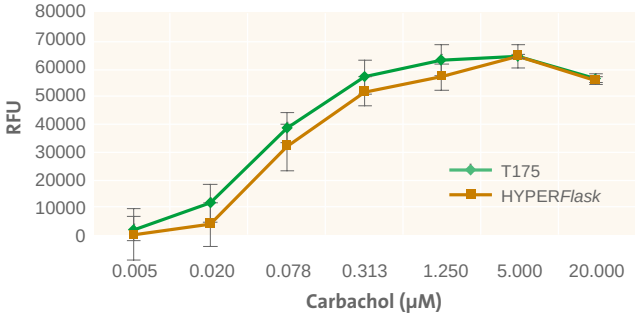


Figure 2. Carbachol Dose Response

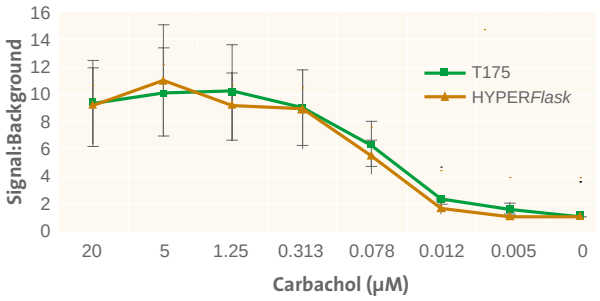


Figure 3. Luciferase Reporter Gene Assay

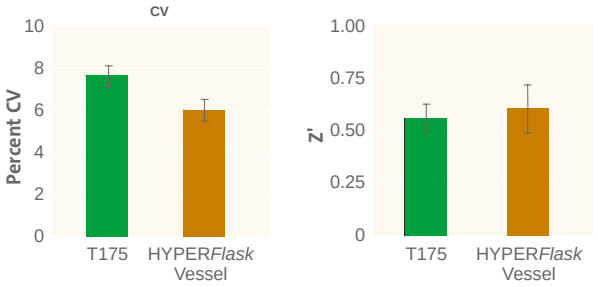
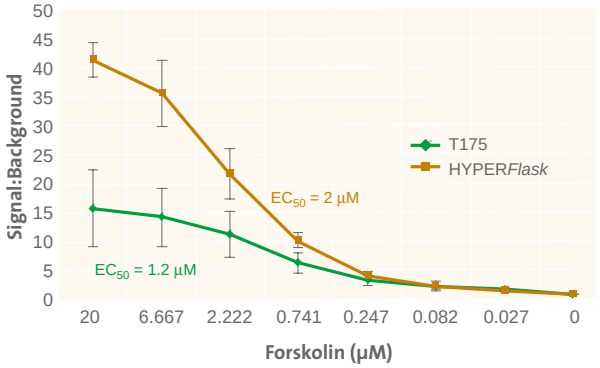


Figure 4. Forskolin Dose Response



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