# Large Scale Growth of Cells for Cell Based Assays Using the Corning HYPERFlask Vessel

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## Introduction

**Abstract**

With the increasing use of cell based assays in drug discovery the requirements for large quantities of cells have risen sharply over the last few years. Those doing screens insist that all reagents, of which cells are a key component, be of high quality and consistency. We previously described the development of a novel flask, called the HYPERFlask, capable of providing ~ 200 million cells in a single easy to handle flask. The current work examines the response and consistency of cell lines grown in the HYPERFlask in comparison to those grown in conventional flasks using a variety of cell based assays. To this end we use a variety of methods to examine GPCR based signaling, including FLIPR assays. Assays run with cells from the HYPERFlask performed as well as or better than cells grown in conventional T175s as evidenced by higher S:B ratios, better CVs and more consistent Z's. Our results indicate that a variety of cell lines grow well in the HYPERFlask and that these cells are excellent reagents for use in cell based assays.

**Corning® HYPERFlask Cell Culture Vessel Advantages:**

- Greater consistency of cells and assays
- Reduced processing and handling time
- 10 fold reduction in space utilization and disposal requirements

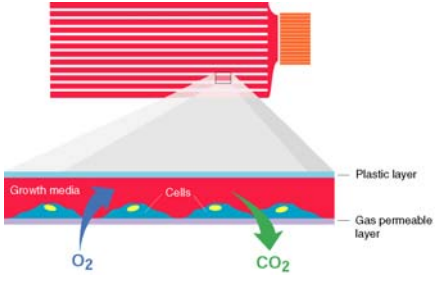



**Figure 1: Corning® HYPERFlask Cell Culture Vessel Principal of Operation.** Ten equivalent layers each containing the same surface area of a gas permeable material are joined together to form a multi-layered cell culture vessel. Cells attach and grow on the gas permeable material with gas exchange occurring through this layer. All fluid manipulations occur through the single neck which is contiguous with all layers.

## Materials & Methods/Results

**Materials**

**Cells:** HEK 293 cells were purchased from ATCC and HEK Cre-Luc were purchased from Panomics.

**Media:** IMDM (Mediatech) with 10% fetal bovine serum (Cambrex) was used as basal media for HEK-293 cultures. IMDM at 10% FBS with 0.1 mg/ml hygromycin B (Invitrogen) was used as basal media for HEK Cre-Luc cultures. IMDM without phenol red (Invitrogen) at 2% FBS was used for assays with both cell lines.

**Vessels:** T175 with vent caps were used as comparison to the HYPERFlask vessel.

**Methods**

**Cell Culture:** Cells were seeded between 10,000 - 15,000 cells/cm2, allowed to grow for 72 hours at which time the cultures were ~80-90 % confluent. Cultures were harvested using HyQTase® (Hyclone). Cells were counted either by hemocytometer or Coulter Counter Z2. All experiments were conducted at least three times.

**Signaling studies:** HEK-293 cells were plated at 40,000 cells/well in 100 ul/ well using CellBIND 96 well black/clear bottom assay plates (p# 3340) and cultured for 24 hr. Utilizing FLIPR® calcium 3 Assay Kit (Molecular Devices) plates were processed following manufacturers' instructions, ATP was used to generate intercellular calcium response. HEK Cre-Luc cells were plated at 5,000 cells/well in 25ul/ well using a TCT treated 384well white bottom plate (p# 3704) Cultures were induced with forskolin and cultured for 24 hr. Using the steadylite HTS luminescence gene reporter assay kit (PerkinElmer) cells were assayed to quantify luciferase expression from cells harvested from both vessels. Plates were read on the LJL Analyst or FlexStation II 384 analyzers. Each assay was performed at least three times with at least six replicates per condition.

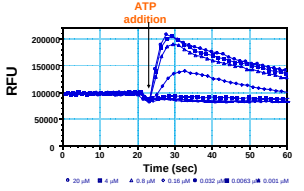



**Figure 2: Kinetics and Assay Window of HEK Cells From HYPERFlask Vessels and T175 Flasks.** HEK cells grown on each vessel were run in calcium flux assays as described. Shown are the kinetics of ATP response for T175 flasks (top left) and HYPERFlask vessels (bottom left). The assay window for cells derived from each vessel type are shown. Cells from both vessels performed identically in this assay.

## Results and Conclusion

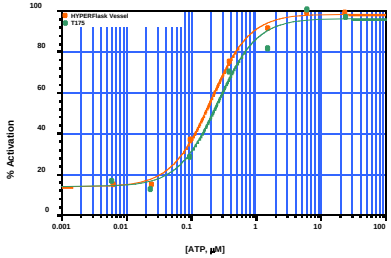
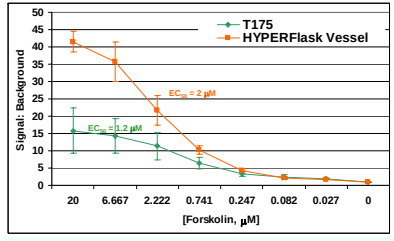



**Figure 3: ATP Dose Response of HEK Cells.** The dose response to ATP of HEK cells was determined for both vessels. Cells from both vessels had similar responses to stimulus with similar $EC_{50}$s.

A

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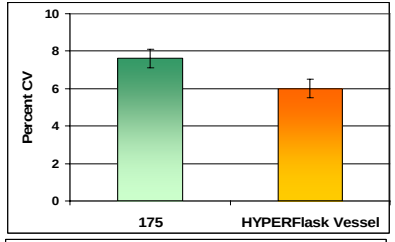


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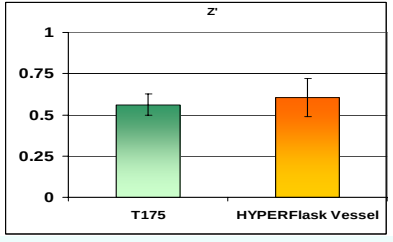


**Figure 5: HEK Cre-Luc Cells Have a Larger Assay Window When Grown in HYPERFlask vessels.** HEK Cre-Luc cells were run in a luciferase reporter assay with cells grown in both vessels. Cells grown in the HYPERFlask had a two fold greater assay window than those grown in a T175 (A). CV was determined for each concentration and averaged to get an overall plate CV (B). Z' values were calculated for each plate in each experiment then averaged across all experiments (C).

## Conclusions:

- Cells grown in the Corning HYPERFlask vessel perform as good as or better than cells grown in a T175 flask in signaling assays such as calcium flux and luciferase reporter assays.
- Assay performance for cells grown in the HYPERFlask vessel is improved for both CV and Z'.
- HEK Cre-Luc cells grown in the HYPERFlask vessel have a an approximate three fold greater assay window than cells grown in T175 flasks.