Label-Free Cell-based Assay for Drug Discovery using the Corning Epic® System and Corning HYPER*Flask*® Cell Culture Vessel

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Introduction

Abstract

The Corning Epic® technology is an optical resonant waveguide-based, label-free detection system. It provides a label-free platform of high detection sensitivity for measuring both molecular interactions in a biochemical assay, as well as integrated cellular responses in a cell-based assay. Detection of live cell and time dependent cellular response in a pathway unbiased manner with the Epic® system may provide previously unattainable biological and pharmacological information for an integrated drug-stimulated cellular response. Moreover, the Epic system is capable of performing primary screening in both biochemical and cell based assays meeting a throughput requirement of approximately 80 384-well microplates/8 hr run. To support the demand of cells for HTS cell-based assays, the Corning HYPER*Flask*® vessel provides a solution with high capacity and high efficiency for cell culture with each HYPER*Flask*® vessel providing the number of cells equivalent to that from 10 standard T-175 flasks. The study reported here describes the successful use of the Epic system for a label-free screening to discover agonists targeting serotonin receptors in a cell-based GPCR assay. It is also demonstrated that the cells cultured in the conventional T-flask and those in the HYPER*Flask*® vessel show highly similar assay performance, thus further supporting the use of HYPER*Flask*® vessel for HTS cell-based assays.

Background

The Corning Epic® technology provides a label-free detection system that measures integrated cellular response in a cell-based assay or molecular interaction response in a biochemical assay. It enables high throughput screening for drug discovery in a label-free format with robust signal detection and sensitivity. Cell-based high throughput screens require a large supply of cells that often present challenges for conventional cell culture techniques. Conventional cell culture requires numerous T-flasks, significant incubator space and a high demand for skilled labor, resulting in substantial costs. The Corning HYPERFlask® vessel provides a solution to the need of high capacity cell culture in support of cell-based HTS screen with its novel design. We present here a comparative study of Epic® assay performance using cells cultured in a HYPERFlask® vessel and conventional T-150 flasks. The Epic® results demonstrated highly similar performance with cells cultured in the HYPERFlask® vessel and in conventional T-flasks. Results of this study showed a significant savings in materials, time and labor costs with equivalent assay performance.

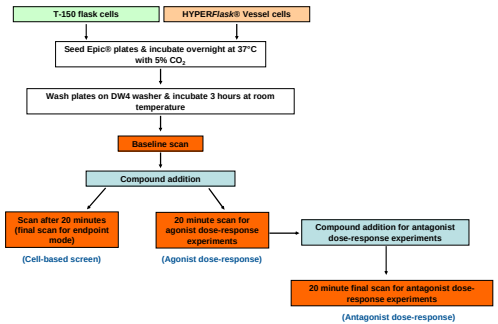
Materials & Methods

Cell Seeding
- HYPERFlask vessels and T-150 flasks were seeded with 1x10^4 cells/cm^2 with engineered CHO cells
- Flasks were incubated at 37°C with 5% CO_2
- Cells were grown for 72 hours at which time they were ~ 90% confluent

Harvesting:
- Cells were harvested using Trypsin-EDTA
- Cell Yields:
  - HYPER*Flask*® vessel: 2.6x10^8 cells per vessel (1.5x10^5 cells/cm^2)
  - T-150 Flask: 0.22x10^8 cells per flask (1.5x10^5 cells/cm^2)

Plate Seeding:
- Fibronectin-coated Epic® microplates were seeded with 7,500 cells/well using a Multi-Drop Combi

Assay Flowchart



Results

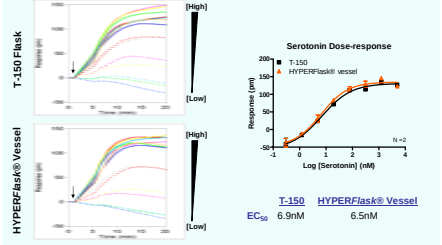


Figure 1: Dose-dependent response of engineered CHO cells stimulated with serotonin. Left panels: Epic® response profiles for engineered CHO cells stimulated with a dilution series of serotonin. Serotonin concentration range: 0.3nM – 5µM. Arrow indicates the point of compound addition.
Right panel: Dose-response curves and EC_{50} values from Epic response profiles.

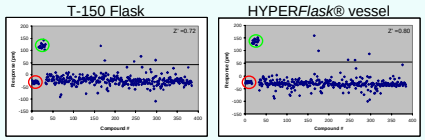


Figure 2: Representative cell-based screening data for engineered CHO cells cultured on a T-150 flask or HYPERFlask® vessel.
Compounds were screened at 5µM. **Left panel:** Scatter plot for cells from T-150 flasks. **Right panel:** Scatter plot for HYPERFlask® vessels. **Red circle:** Negative control (assay buffer, N=16) **Green circle:** Positive control (Serotonin, EC_{95} concentration, N=16). The threshold was set at 40% of the response of the positive control. The same 5 compounds were identified as hits on both platforms. Z' > 0.7

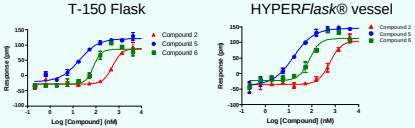


Figure 3: Agonist dose-response curves for a subset of the compounds that were identified as hits during the cell-based screen.
A dilution series of each compound was added to the cells and the response was measured at 20 minutes. Concentration range: 0.2nM – 12.5µM

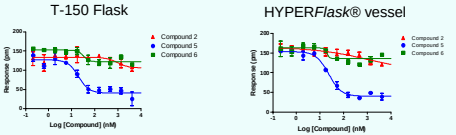


Figure 4: Inhibition curves for a subset of the compounds that were identified as agonist hits during the cell-based screen.
A dilution series of each compound was added to the cells and the cells were incubated for ~30 minutes. An EC_{95} concentration of serotonin was added to the cells and the response was measured after an additional 20 minutes. Compound #5 inhibits the serotonin response most likely due to receptor desensitization. Compounds #2 and #6 do not inhibit the serotonin response. These compounds may be targeting other endogenously expressed receptors on the engineered CHO cells (not the serotonin receptor).

Table 1: Summary of EC_{50} and IC_{50} values measured on the Epic® System

Compound	EC_{50} (nM) T-150	EC_{50} (nM) HYPERFlask® *Vessel*
1	5	10
2	539	578
3	1006	964
4	126	76
5	19	16
6	78	77
7	>10000	>10000
8	436	480
9	800	1006
10	776	1154
11	352	417
12	>10000	>10000
13	233	309
14	54	57
15	375	570
16	585	530

Compound	IC_{50} (nM) T-150	IC_{50} (nM) HYPERFlask® *Vessel*
1	5	10
2	No inhibition	No inhibition
3	No inhibition	No inhibition
4	No inhibition	No inhibition
5	20	23
6	No inhibition	No inhibition
7	No inhibition	No inhibition
8	382	705
9	No inhibition	No inhibition
10	No inhibition	No inhibition
11	146	264
12	2491	3248
13	No inhibition	No inhibition
14	30	43
15	No inhibition	No inhibition
16	No inhibition	No inhibition

- EC_{50} and IC_{50} values were calculated using GraphPad Prism.
- Similar EC_{50} and IC_{50} values were observed for the all compounds tested.
- Dose-response curves for compounds 2, 5, and 6 are shown in Figures 3 and 4.

Summary and Conclusions:

- Cells cultured on the HYPER*Flask*® vessel and a T-150 flask exhibited similar morphology and Epic assay performance.
- Epic response profiles, EC_{50} values and assay robustness (Z'>0.7) for cells from both vessels are in good agreement.
- The same compounds were identified as hits during the agonist cell-based screen with both sets of cells.
- The HYPER*Flask*® vessel can be used to replace multiple T-flasks for cell-based high throughput screening with no compromise to the results of the assay.
- Using the HYPER*Flask*® vessel will:
  - Increase cell yield
  - Generate less waste
  - Decrease labor costs
  - Require less incubator space
- Results demonstrated that Epic can be used as a robust label-free screening tool for high throughput drug discovery.
- The combination of the HYPER*Flask*® vessel and Epic provides a highly efficient and robust platform for high throughput cell-based screening in a label-free format.