

# Corning® Epic® Label-Free Cell Based Assay: Development of a One-Day Assay for Adherent Cells

CORNING

Epic<sup>®</sup>  
technology



## SnAPPShots

A brief technical report  
from the Corning  
Applications Group

*Louie Tran, Kristine Krebs,  
Todd Upton, and Alice Gao  
Corning Incorporated,  
Life Sciences  
Kennebunk, ME 04043*

## Introduction

The Corning Epic technology permits high-throughput, label-free detection in both cell-based and biochemical assays. The Epic system consists of an ANSI/SBS 384 well microplate with integrated optical biosensors and a microplate reader that can be integrated with other high-throughput screening equipment. The Epic technology gives insight into global events caused by changes in cell morphologies and mass redistribution of cellular contents within the cells (1). It is known that activation of receptors leads to the dynamic translocation of multiple signaling molecules or molecular assemblies during its signaling cycle. Such movement and/or reorganization results in dynamic redistribution of cellular contents termed dynamic mass redistribution (DMR), which can be monitored using the optical biosensors. The resultant DMR signal offers a novel and functional optical signature for studying cellular signaling and screening of drug compounds.

For adherent cell lines, it is common to seed the cells the day before performing assays and to allow the cells to attach, spread out and recover overnight. Thus, it would require

2 days to generate assay results. The following demonstrates that cell seeding and assaying can be accomplished on the same day, thus reducing the time needed to generate results. In this study, we evaluated a loosely adherent cell line that endogenously expresses the muscarinic and adrenergic receptors (HEK 293) and two strongly adherent cell lines that express recombinant opioid receptor (CHO-OP3) or muscarinic M1 receptor (CHO-M1WT2).

## Materials and Methods

### Cells

HEK 293 (Cat. No. CRL-1573) and CHO-M1WT2 (Cat. No. CRL-1984) were purchased from American Type Culture Collection, (Manassas, VA). CHO-OP3 (Cat. No. ES-542-C) was obtained from PerkinElmer (Waltham, MA). All cell culture reagents were purchased from Invitrogen (Carlsbad, CA). CHO-M1WT2 and CHO-OP3 were cultured in F12-K medium supplemented with 10% heat inactivated fetal bovine serum (Cat. No. 10082). HEK 293 was cultured in Dulbecco's Modified Eagle Medium (Cat. No. 10313-039) supplemented with 10% heat inactivated fetal bovine serum and 1% L-glutamine (Cat. No. 25030-081). Assay buffer was Hank's Balanced Salt Solution (HBSS) (Cat. No. 14025) containing 20 mM HEPES (Cat. No. 15630), 0.05% bovine serum albumin (BSA) and 1% dimethyl sulfoxide (DMSO).

### Reagents

D-Ala<sup>2</sup>, N-Me-Phe<sup>4</sup>, Gly<sup>5</sup>-ol]-Enkephalin acetate salt (DAMGO) (Cat. No. E7384) and Epinephrine (Cat. No. E4642) were purchased from Sigma Aldrich (St. Louis, MO) and Acetylcholine chloride (ACh) (Cat. No. 2809) was purchased from Tocris (Ellisville, MS).

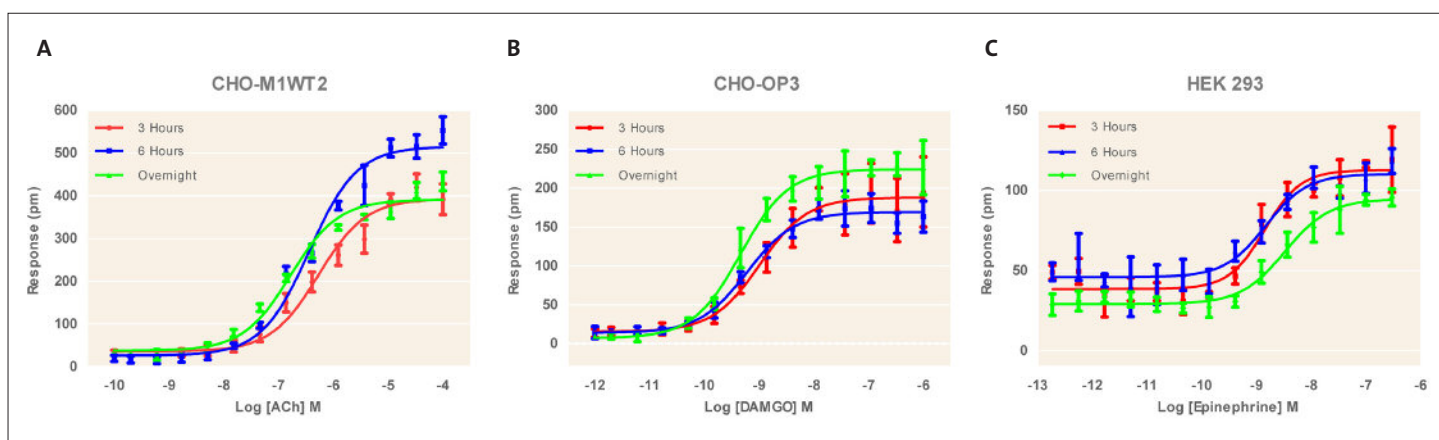
## Assay Procedure

Harvested cells were resuspended in the recommended culture medium and enumerated. Cells were then seeded into an Epic® 384 well fibronectin-coated microplate (Corning® Cat. No. 5042). The cell seeded microplates were allowed to rest at room temperature for 30 minutes before being placed in a 37°C, 5% CO<sub>2</sub> humidity-controlled incubator. After a specified period of time (from 3 hours to overnight), growth medium was replaced with assay buffer, leaving a final volume of 40 µL assay buffer per well. The microplates were then placed inside the Epic reader and allowed to equilibrate for 1.5 to 2 hours. Following this equilibration step, baseline measurements were obtained for 5 minutes. After baseline measurements, 10 µL of compound was added to the cells, mixed 3 times and kinetic measurements were taken for 50 minutes. Once the assay was completed, the kinetic profiles were analyzed using the Epic software, and dose-response curves were generated using GraphPad Prism® software. The response difference between the maximum signal after compound addition and the first data from the baseline measurements was used in the calculations.

## Results and Discussion

In order to determine the minimal incubation time required for the cells to become assay-ready after seeding, cells were seeded at 6,000 cells per well for CHO cells and 15,000 cells per well for HEK cells and incubated for 3 hours, 6 hours or overnight prior to being assayed. Seeding densities were optimized for assays with overnight cultures of each respective cell line. The agonist dose-response curves for all three cell lines or targets tested show different incubation times and gave very similar results (Fig. 1). The EC<sub>50</sub> for all of the incubation times are within 3-fold of each other, and the assay windows were comparable (Table 1). In addition to having similar EC<sub>50</sub> values, the DMR profiles also remained the same after the different incubation times (Fig. 2). Therefore, it is concluded that a 3-hour incubation time in a 37°C, 5% CO<sub>2</sub> humidity-controlled incubator post-seeding was sufficient for these cells to become assay ready. This reduced incubation time allows the user to complete an assay within an 8-hour work day without compromising data quality.

Following the incubation time study, various cell seeding densities were evaluated to determine the optimal number



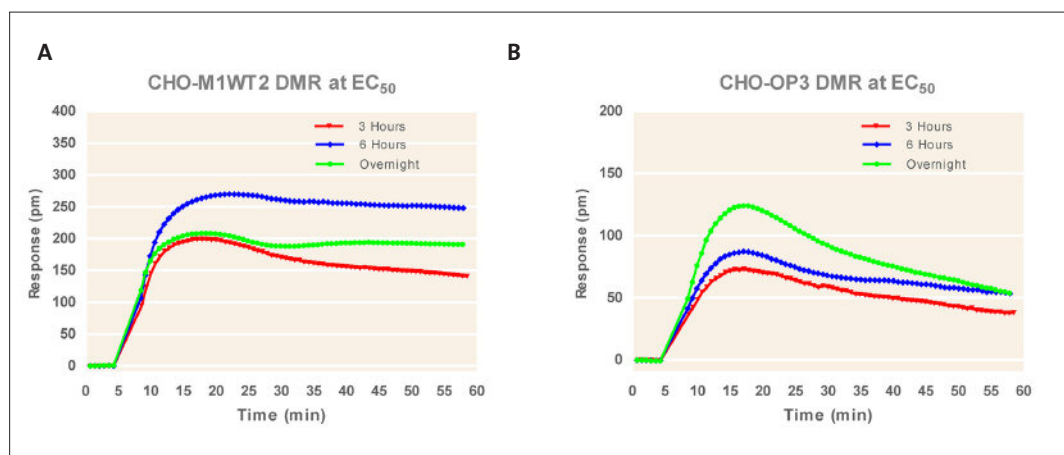
**Figure 1.** Dose response curves for CHO-M1WT2, CHO-OP3 and HEK 293 at various incubation times after seeding. The red, blue and green traces represent 3 hours, 6 hours and overnight incubation after seeding, respectively. (A) ACh agonist response on CHO-M1WT2 cells. (B) DAMGO agonist response on CHO-OP3 cells. (C) Epinephrine agonist response on HEK 293 cells.

**Table 1. Summary of agonist pharmacology (EC<sub>50</sub>) and assay window for CHO-M1WT2, CHO-OP3 and HEK 293 cells at various seeding incubation times.**

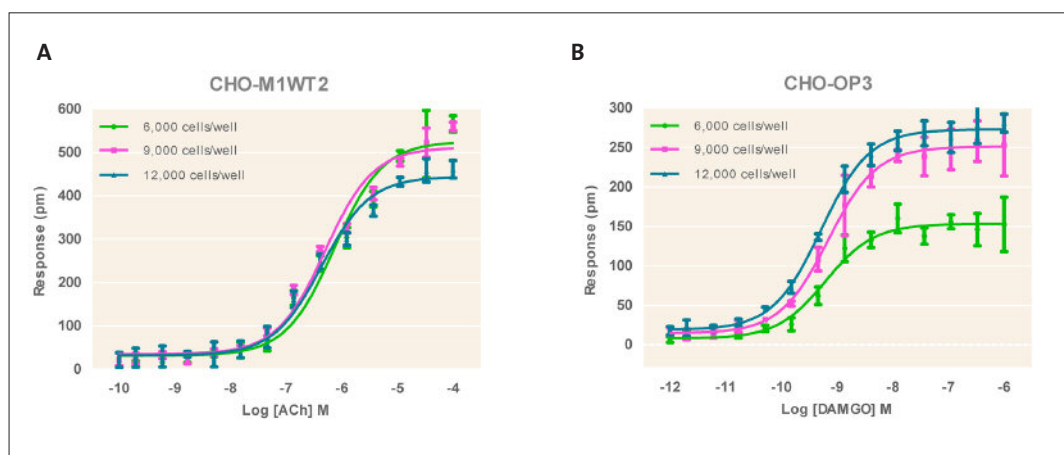
Seeding Incubation Time	CHO-M1WT2 ACh		CHO-OP3 DAMGO		HEK 293 Epinephrine	
	EC <sub>50</sub> (µM)	Assay Window (pm)	EC <sub>50</sub> (nM)	Assay Window (pm)	EC <sub>50</sub> (nM)	Assay Window (pm)
3 Hours	0.54	357	1.05	173	1.42	74
6 Hours	0.36	489	0.59	156	1.48	64
Overnight	0.17	353	0.46	217	3.33	66

of cells needed to obtain maximal cellular responses when incubation times were shortened to 3 hours from overnight. For both CHO cell lines, seeding densities of 6,000, 9,000 and 12,000 cells per well were tested, and for HEK 293, 15,000, 20,000 and 25,000 cells per well were tested. It was found that the impact of seeding density varies depending on the cell line or target. As indicated by Figs. 3 and 4 and

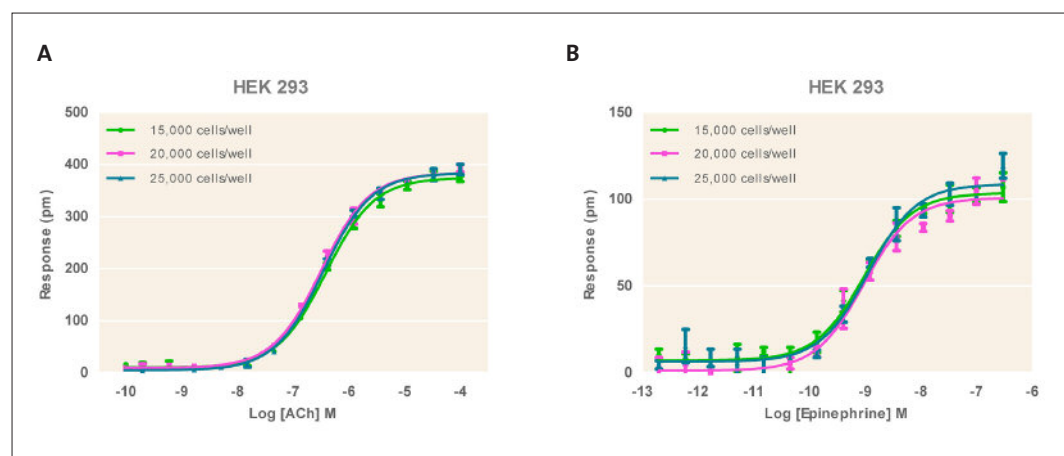
Table 2, increasing cell seeding density did not increase or decrease the assay window for CHO-M1WT2 and HEK 293. However, increasing cell seeding density did improve the assay window for CHO-OP3. Therefore, the optimal seeding density for CHO-M1WT2, CHO-OP3 and HEK 293 is 6,000, 9,000 and 15,000 cells per well, respectively, when a 3-hour incubation time post-seeding is used.



**Figure 2.** DMR profiles for CHO-M1WT2, CHO-OP3 and HEK 293 cells. The red, blue and green traces represent 3 hours, 6 hours and overnight incubation after seeding, respectively. (A) ACh DMR profile at EC<sub>50</sub> on CHO-M1WT2. (B) DAMGO DMR profile at EC<sub>50</sub> on CHO-OP3. (C) Epinephrine DMR profile at EC<sub>50</sub> on HEK 293.



**Figure 3.** Dose response curves for CHO-M1WT2 and CHO-OP3 cells seeded at various cell densities and incubated for 3 hours after seeding. The green, magenta and light blue traces represent cells seeded at 6,000, 9,000 and 12,000 cells per well, respectively. (A) ACh agonist response on CHO-M1WT2. (B) DAMGO DMR profile at EC<sub>50</sub> on CHO-OP3.



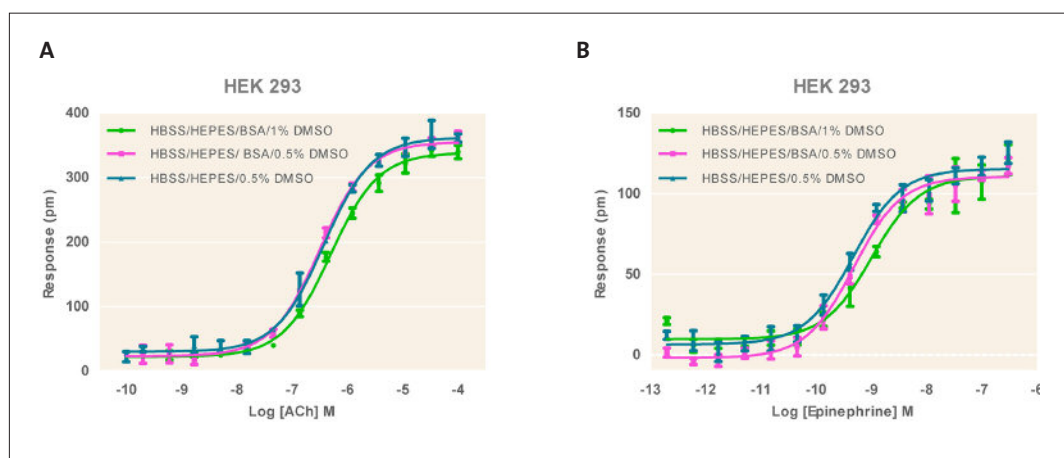
**Figure 4.** Dose response curves for HEK 293 cells seeded at various cell densities and incubated for 3 hours after seeding. The green, magenta and light blue traces represent cells seeded at 15,000, 20,000 and 25,000 cells per well, respectively. (A) ACh agonist response. (B) Epinephrine agonist response.

Finally, the tolerance to DMSO and BSA using HEK 293 cells in assays with 3-hour post-seeding incubation time was evaluated. The results show that increasing DMSO concentrations up to 1%, with or without BSA had no effect on the dose response curves for ACh and Epinephrine stimulat- ed responses (Fig. 5). The EC<sub>50</sub> for different DMSO and

BSA concentrations were within 3-fold of each other, and the assay windows were comparable (Table 3). This suggests that the assay using HEK 293 cells with reduced incubation time after seeding can tolerate DMSO up to 1% and BSA in the assay buffer.

**Table 2. Summary of agonist pharmacology (EC<sub>50</sub>) and assay window for CHO-M1WT2, CHO-OP3 and HEK 293 at various seeding densities.**

Cells per well	CHO-M1WT2		CHO-OP3		HEK 293			
	ACh		DAMGO		ACh		Epinephrine	
	EC <sub>50</sub> (μM)	Assay Window (pm)	EC <sub>50</sub> (nM)	Assay Window (pm)	EC <sub>50</sub> (μM)	Assay Window (pm)	EC <sub>50</sub> (nM)	Assay Window (pm)
6,000	0.73	494	0.61	145				
9,000	0.50	477	0.69	237				
12,000	0.44	412	0.52	254				
15,000					0.39	364	0.96	97
20,000					0.32	375	0.95	100
25,000					0.35	379	1.14	102



**Figure 5.** Dose response curves for HEK 293 cells seeded at 15,000 cells per well and incubated for 3 hours after seeding. The green, magenta and light blue traces represent cells assayed in assay buffer with 1% DMSO, assay buffer with 0.5% DMSO, and assay buffer with 0.5% DMSO and without any BSA, respectively. (A) ACh agonist response. (B) Epinephrine agonist response.

**Table 3. Summary of agonist pharmacology (EC<sub>50</sub>) and assay window for HEK 293 when assayed with varying DMSO and BSA concentrations.**

	HEK 293			
	ATCh		Epinephrine	
Assay Buffer	EC <sub>50</sub> (μM)	Assay Window (pm)	EC <sub>50</sub> (nM)	Assay Window (pm)
Assay Buffer	0.49	316	1.01	101
Assay Buffer with 0.5% DMSO	0.32	332	0.48	112
Assay Buffer with 0.5% DMSO and without BSA	0.37	332	0.47	109

## Conclusions

- ▶ A single day assay was developed on the Epic® system for three different adherent cell lines that are CHO- or HEK 293-derived.
- ▶ Reduced incubation time after seeding from overnight to 3 or 6 hours gave similar assay results to that obtained with standard overnight cultures. Therefore, 3-hour incubation time post seeding is recommended for 1-day Epic cell assays.

- ▶ The effect of cell seeding density with the shortened incubation period varies depending on cell lines or targets; therefore, it is recommended as part of the assay optimization test for each cell line to evaluate cell seeding densities.
- ▶ It was demonstrated that reduced incubation time did not affect the ability of the HEK 293 cells to tolerate DMSO and BSA at the concentrations tested.

## Reference

1. Fang, Y., et al. Resonant waveguide grating biosensor for living cell sensing. *Biophys. J.* 91(5):1925-1940 (2006).

# 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](http://www.corning.com/lifesciences)

## **Worldwide Support Offices**

### **ASIA / PACIFIC** **Australia/New Zealand**

t 0402-794-347  
**China**  
t 86 21 2215 2888  
f 86 21 6215 2988

**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 2-2516-7500

## **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