

# Impact of Cell Plating Medium on Corning® Epic® Cell-Based Assay Performance

## Application Note

CORNING  
Epic<sub>technology</sub>

Alice Gao, Ph.D.  
Corning Incorporated  
Life Sciences  
Kennebunk, ME 04043

### Introduction

Epic label-free cell assay technology utilizes a resonant waveguide grating sensor to monitor molecular network interactions<sup>1</sup> and has been successfully used to measure cellular activities associated with the activation of cell surface targets, such as G-protein coupled receptors, ion channels and receptor tyrosine kinases.<sup>2-4</sup> These assays not only provide a wide range of benefits, such as no requirement for labels and sufficient sensitivity to monitor the activity of many cellular receptors at endogenous levels, they also do not require complex assay procedures. In general, cells in growth medium are seeded onto specially fabricated microplates containing biosensors at the bottom of each well. After overnight culturing at 37°C, growth medium is replaced with assay buffer. Cellular activities before and after exposure to a stimulus, such as molecular mass movements, changes in cell adherence, cell shape and volume, are then recorded kinetically as changes in wavelength shift measured in picometers.

In this study, the performance of Epic cell-based assays using standard growth medium with normal or reduced amounts of serum, and serum-free medium during the cell seeding step was compared. It was demonstrated that serum-free medium can significantly improve assay performance.

### Materials and Methods

#### Reagents

Mu-Opioid receptor agonist DAMGO and antagonist CTOP were purchased from Sigma-Aldrich (St. Louis, MO) and Tocris Bioscience (Ellisville, MO), respectively. Agonists for the 5HT<sub>2C</sub> receptor serotonin and  $\alpha$ -me-serotonin, as well as antagonists Methysergide and Ketanserin were purchased from Sigma-Aldrich. CP94253 and SP224289 were obtained from Tocris Bioscience. All cell culture reagents and assay buffer components were purchased from Invitrogen (Carlsbad, CA), except for the UltraCHO medium which was obtained from Lonza (Cat. No. 12-724Q, Walkersville, MD). Corning polypropylene microplates (Cat. No. 3657) were used to pre-

pare ligand solutions and fibronectin-coated Corning Epic microplates (Cat. No. 5042) were used for all assays performed in this study.

#### Cell lines

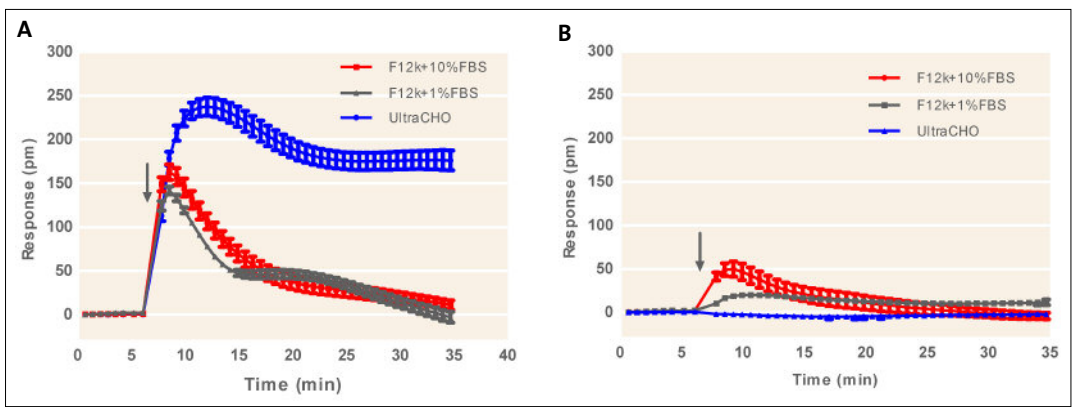
The two cell lines used in this study were kindly provided by PerkinElmer. They were recombinant chinese hamster ovary (CHO) cells stably expressing mu-Opioid (OP<sub>3</sub>) receptor (Cat. No. ES-542-C) or 5HT<sub>2C</sub> receptor (Cat. No. ES-318-A). OP<sub>3</sub>-expressing CHO cells were grown and maintained in F12K medium containing 10% heat-inactivated fetal bovine serum (FBS), 1% Pen/Strep, and 400  $\mu$ g/mL G418 as recommended by the cell supplier. 5HT<sub>2C</sub>-expressing CHO cells were maintained in UltraCHO medium containing 1% dialyzed FBS and 1% Pen/Strep as recommended by the cell supplier.

#### Epic Assay Procedures

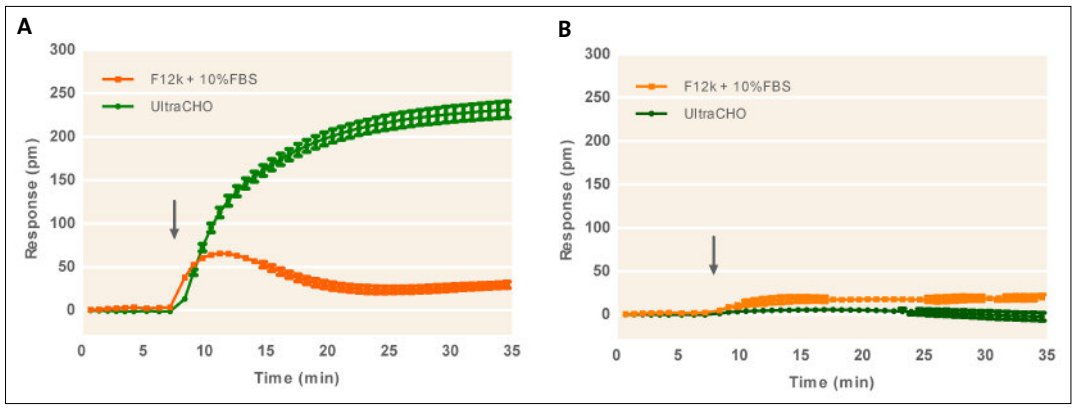
One day prior to performing the assay, fresh cells in flasks were trypsinized and harvested by centrifugation at 800 rpm (130 x g) for 3 minutes. The cell pellets were then resuspended in seeding medium. The resulting cell suspensions were used to seed Epic cell assay microplates at 8,000 cells per well in a 40  $\mu$ L volume. Seeding media were either F12K containing 10% or 1% FBS or UltraCHO without any FBS. After seeding, microplates were allowed to sit in a laminar flow hood for 30 minutes before being placed in a humidity-controlled CO<sub>2</sub> incubator at 37°C. After overnight incubation, the media in assay microplates was replaced with assay buffer (HBSS containing calcium, magnesium, 20 mM HEPES, 0.05% fatty acid free BSA and 1% DMSO). The microplates were allowed to equilibrate inside the Epic reader for 1 to 2.5 hours. Baseline signals were then measured followed by the addition of test ligands. The dynamic mass redistribution (DMR) response to ligand addition was monitored immediately for 30 to 40 minutes. In the assays involving antagonists, cells were pre-incubated with antagonists for 30 minutes prior to baseline measurement.

#### Data Analysis

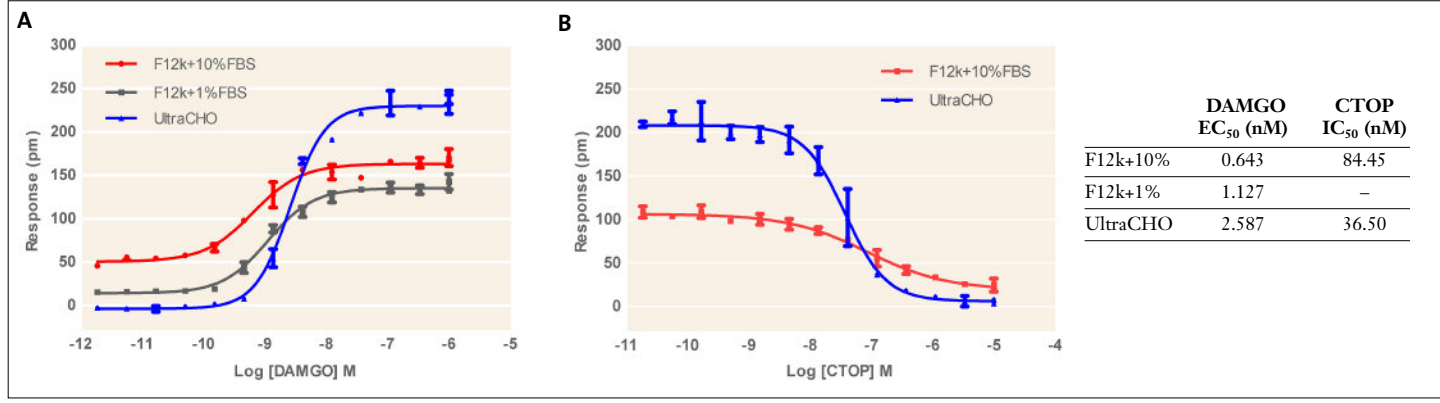
The response profiles/traces were obtained using Epic Offline Viewer software. Dose responses and curve fittings were constructed using GraphPad Prism® software.



**Figure 1.** DMR response profiles of mu-Opioid ( $OP_3$ )-expressing CHO cells following stimulation with 100 nM of the reference agonist DAMGO (A) or buffer (B). Each curve in the graphs represents the response from cells seeded in different media, F12K + 10% FBS, F12K + 1% FBS, or UltraCHO. Arrows indicate the addition of the agonist or buffer (N = 4).



**Figure 2.** DMR response profiles of 5HT<sub>2C</sub>-expressing CHO cells following stimulation with 300 nM of the reference agonist serotonin (A) or buffer (B). Each curve in the graphs represents the response from cells seeded in F12K + 10% FBS or UltraCHO. Arrows indicate the addition of the agonist or buffer (N = 4).



**Figure 3.** Comparison of agonist and antagonist pharmacology assayed with  $OP_3$ -expressing CHO cells seeded in different media. Values used here were obtained from the peak signals occurring about 3 minutes after DAMGO stimulation for cells seeded in standard growth medium and at ~7 minutes after DAMGO stimulation for cells seeded in UltraCHO medium (N = 4).

**Results and Discussions**

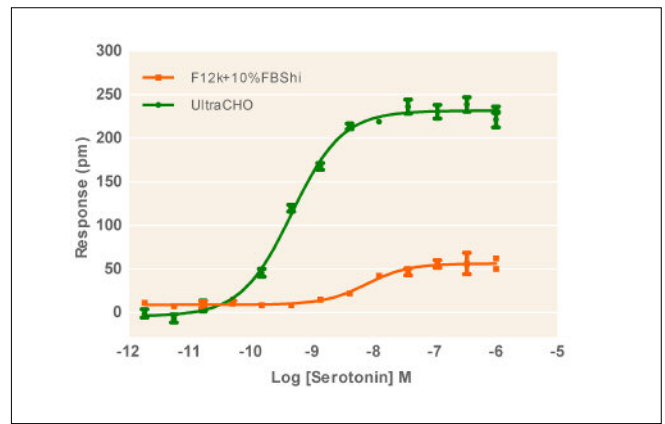
**Comparison of Receptor Response Profiles and Assay Window**

The effect of cell culture medium on the DMR kinetic profiles of  $OP_3$ -expressing cells stimulated with DAMGO is shown in Fig. 1. As shown, when cells were seeded in F12K containing 10% FBS, the DMR response quickly reached a peak of 170 pm after stimulation and then declined close to baseline after 40 minutes. The DMR response from cells seeded in UltraCHO medium reached a higher peak of 250 pm about 2 minutes after stimulation and then maintained the response at about 180 pm. Cells seeded with F12K containing only 1% FBS behaved similarly to the

cells seeded in F12K containing 10% FBS with a slightly reduced maximal response (Fig. 1A). Buffer addition induced a significant DMR response of ~50 pm from cells seeded in F12K with 10% FBS, but almost none from cells seeded in UltraCHO medium or F12K with 1% FBS (Fig. 1B). Similar effects and trends were observed in 5-HT<sub>2C</sub>-expressing CHO cells (Fig. 2). However, differences in both the magnitude and timing of the peak response to serotonin were more dramatic between the cells in the two seeding media. It is known that serum contains reasonable levels of serotonin; therefore, seeding cells with medium containing a large amount of FBS could cause the receptor to become desensitized and eventually down-regulate the receptor expression level.

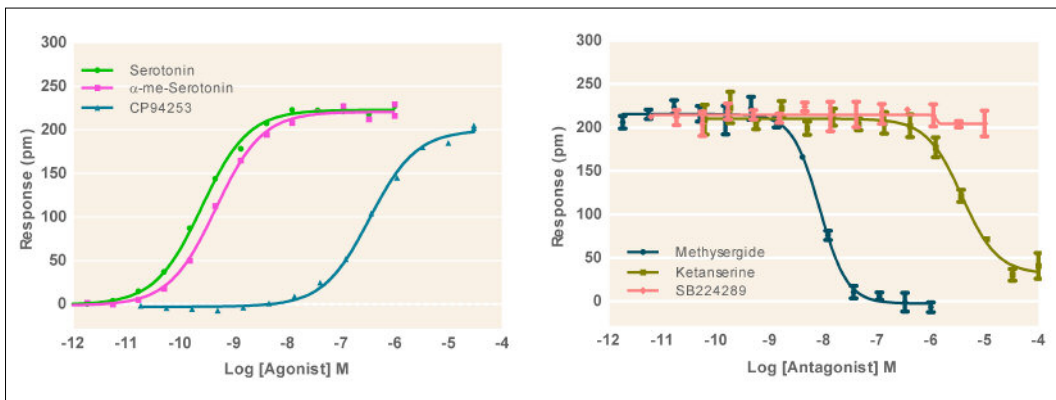
## Impact to Ligand Pharmacology

A comparison of agonist and antagonist pharmacology to evaluate the effect of seeding medium is illustrated in Fig. 3, 4 and 5. Overall, no significant differences were observed whether F12K with 10% FBS or UltraCHO medium was used in cell seeding. For example, agonist potency for DAMGO was only slightly right shifted (within 5 fold), while antagonist potency for CTOP was slightly left-shifted (Fig. 3). Serotonin potency for 5HT<sub>2C</sub> target obtained using UltraCHO as seeding medium was in range with the values from the product literature supplied by PerkinElmer and was about 10-fold more potent compared to that obtained using seeding F12K containing 10% FBS (Fig. 4). This is likely due to the fact that CHO cells endogenously express the 5HT<sub>1B</sub> receptor which signals through the G<sub>i</sub> pathway. The endogenous 5HT<sub>1B</sub> receptor response to serotonin also peaks about 5 to 10 minutes after stimulation (data not shown), which coincides with the peaking time of 5HT<sub>2C</sub> activity in cells seeded in medium containing 10% FBS. As a result, the peak signal at 6 minutes, which was used to construct the dose curve for 5HT<sub>2C</sub> activity under these assay conditions likely reflects the dual activity of both the over-expressed 5HT<sub>2C</sub> and the endogenous 5HT<sub>1B</sub> activity, leading to altered pharmacology. When seeding in UltraCHO medium, higher signals were obtained 30 minutes after

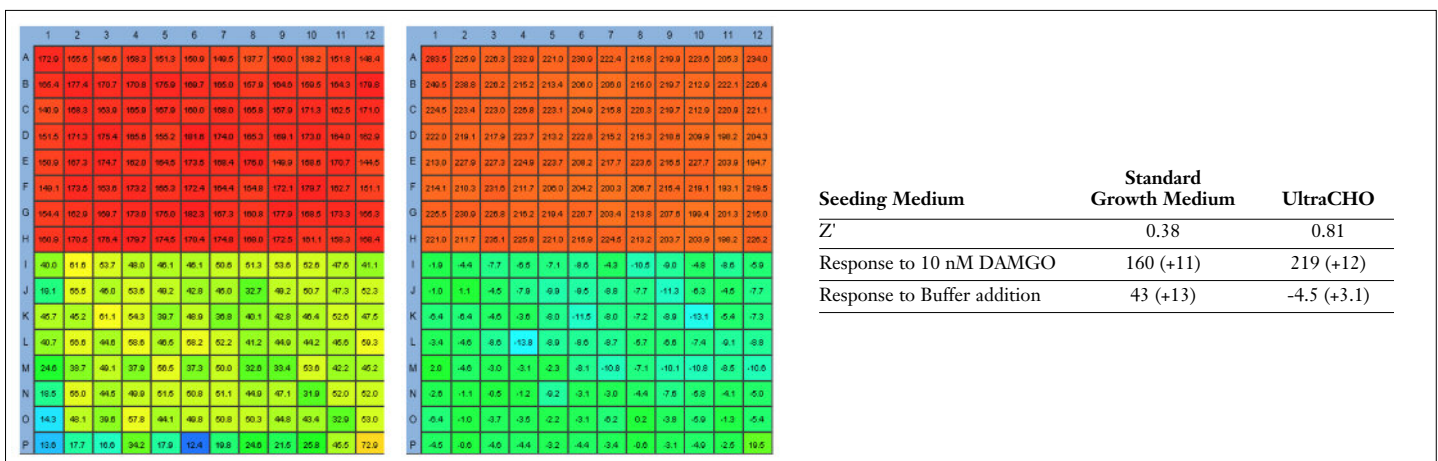


**Figure 4.** Impact of seeding medium on agonist pharmacology using 5HT<sub>2C</sub>-expressing CHO cells. Values used here were obtained from the peak signals occurring about 6 minutes after serotonin stimulation for cells seeded in F12K with 10% FBS and at 30 minutes after serotonin stimulation for cells seeded in UltraCHO medium. Serotonin EC<sub>50</sub> was 0.47 nM for cells seeded in UltraCHO medium and 7.76 nM for cells seeded in F12K with 10% FBS (N = 4).

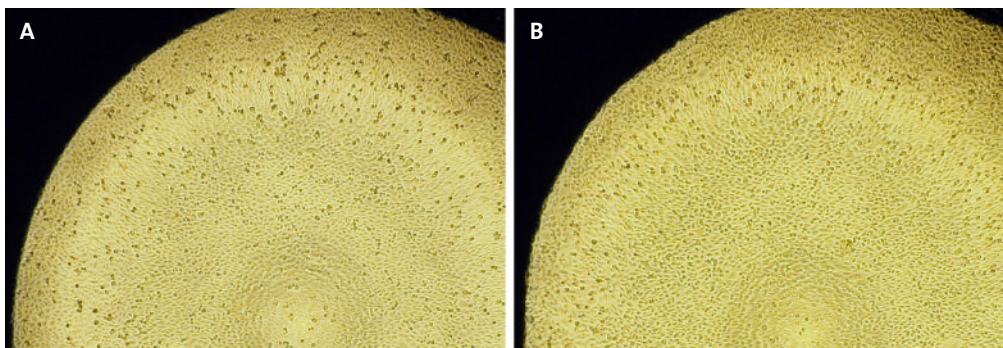
serotonin stimulation, thus interference from 5HT<sub>1B</sub> activity is likely to be absent. The specificity of 5HT<sub>2C</sub> activity measured at 30 minutes after serotonin stimulation was demonstrated by using 5HT<sub>1B</sub> specific antagonist SB224289,



**Figure 5.** Agonist and antagonist pharmacology with 5HT<sub>2C</sub>-expressing CHO cells seeded in UltraCHO medium. EC<sub>50</sub> values for serotonin,  $\alpha$ -me-serotonin and CP94253 were 0.4 nM, 0.5 nM and 367 nM, respectively. IC<sub>50</sub> values for Methysergide and Ketanserin were 8.6 nM and 3819 nM, respectively. SB224289 is a 5HT<sub>1B</sub>-specific antagonist and did not show any inhibition to serotonin-induced 5HT<sub>2C</sub> activity (N = 4).



**Figure 6.** Impact of seeding serum on assay robustness with mu-Opioid-expressing CHO cells. Top quadrants of the plate were stimulated with 10 nM DAMGO which was near EC<sub>95</sub>, while bottom quadrants were added with buffer. (A) Cells were seeded in standard growth medium. (B) Cells were seeded in UltraCHO medium.



**Figure 7.** Microscopic observation of mu-Opioid-expressing CHO cell cultures seeded in standard growth medium (A) or in UltraCHO medium (B). No distinct difference was observed.

which showed no inhibition to the signal (Fig. 5B). The current study also showed that when seeding in UltraCHO medium, the potency values and ranking of other 5HT<sub>2C</sub> agonists and antagonists (see Fig. 5) were also in agreement with the values from the product literature supplied by PerkinElmer.

### Comparison of Assay Robustness

The impact of seeding medium in assay robustness is evident. As shown in Fig. 6, when seeding in F12K with 10% FBS, a significant edge effect was observed from the buffer controls (Fig. 6A), resulting in signal variation of ~35 pm from the edge/corner wells to the center wells. This large variation in buffer control wells, together with the relatively small assay window, resulted in a marginal Z' of 0.38. No clear edge effect was observed when seeding cells in UltraCHO medium (Fig. 6B). Signal variations for both buffer controls and DAMGO stimulated wells were very small. Coupled with a 25% increase in DAMGO-induced signal, overall assay robustness was dramatically improved with Z' values of greater than 0.8.

Despite these differences in assay performance, cell morphology and confluency, when visualized under the microscope, did not seem to be any different between the cells seeded in F12K with 10% FBS and UltraCHO medium (Fig. 7). This result suggests that the differences observed in the DMR response profiles and the magnitude for cells seeded in different media did not result from cell density, or cell attachment to the well bottom.

### Conclusions

Significant improvements in Epic® cell-based assay performance were observed using UltraCHO medium in the cell seeding step. These improvements included both the increased response magnitude and the reduced non-specific background responses, resulting in significant increase in the assay window. In addition, using UltraCHO medium to plate cells also reduced edge effects dramatically, leading to enhanced robustness of assay performance. Results from compound pharmacology studies demonstrated that this assay condition does not alter the test compound behavior. In summary, UltraCHO medium offers significant advantages that make it the recommended choice of cell seeding medium in optimizing assay performance with targets in the CHO cell background.

### References

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## 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)

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