

# Corning® Epic® Cell-based Assays: Tolerance to DMSO Mismatching in Antagonist Assays



## SnAPPShots

A brief technical report  
from the Corning  
Development Group

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## Introduction

The Epic System permits high-throughput, label-free detection in both cell-based and biochemical assays. The Epic System consists of an ANSI/SBS-standard 384 well microplate with integrated optical biosensors and a high-throughput screening (HTS) compatible microplate reader that can be operated as a standalone device for assay development or integrated with other HTS equipment.

The Epic System gives more insight into the global effect of a compound as the quantitative signal results from cell shape changes and mass redistribution of proteins in the cell (1). Therefore, experiments can be performed using primary cells or transformed cells expressing endogenous levels of receptor. Compounds that bind to extracellular receptors generate downstream signals that result in the movement of proteins towards or away from the biosensor or cell shape changes in the sensing region. This process is called dynamic mass redistribution (DMR).

Label-free detection methods based on the changes in refractive index as a readout must consider matching bulk refractive index in assay buffer. This “matching” ensures that specific changes in cellular events are measured, not changes induced by mismatch of bulk buffer refractive index.

DMSO (Index of refraction:  $N_{20} = 1.4785$ ) is used routinely as a solvent for compound libraries. This is a dense solvent that is capable of eliciting changes not only in the index of refraction but on the cells themselves. For matching of bulk refractive index prior to and after compound addition in standard Epic cell-based assays, cells are washed into assay buffer containing the same concentration of DMSO present in compound additions, usually between 0.1 and 2%.

Compound libraries initially prepared in 100% DMSO may have various levels of DMSO content due to the hygroscopic nature of DMSO, particularly if the storage environment is humid. In addition, pipetting errors in compound reformatting into assay buffers may result in subtle differences in DMSO concentration.

In this study, the tolerance to DMSO mismatching in three cell lines commonly used in HTS was examined: CHO M1, A431 and HEK 293 cells. Experiments were performed with the mismatch in the antagonist addition to the assay. Agonist was then added to the cells in matched conditions, and  $IC_{50}$  values were calculated and compared across a range of DMSO mismatch conditions.

## Materials and Methods

### Cell Culture

CHO M1, HEK 293 and A431 cells were purchased from the ATCC® and grown in the recommended media. Cells were seeded at 12,000, 18,000 and 15,000 cells/well, respectively. A431 cells were seeded into uncoated Epic cell-biology assay microplates (Corning Cat No. 5040), and CHO M1 and HEK 293 cells were seeded into Epic fibronectin-coated microplates (Corning Cat. No. 5042). Cells were left for 30 minutes at room temperature to settle, followed by incubation overnight at 37°C, 5% CO<sub>2</sub>.

### Cell Washing

Cells were washed using a Biomek® NX pipettor (Beckman Coulter®). Washing was carried out twice with 50 µL of assay buffer (20 mM Hepes, HBSS, 1% DMSO). The final volume in each well was 40 µL.

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Epic<sup>®</sup>  
system

## Assay Details

Epic® microplates were incubated for 1.5 hours on the carousel of the Epic instrument for temperature equilibration. A baseline reading was taken, and then 10  $\mu\text{L}$  of antagonist compound (Table 1) was added at a dispensing speed of 8.3  $\mu\text{L}/\text{second}$  containing different percentages of DMSO (0.5, 0.75, 1 and 2%). Kinetic measurements were taken for 20 to 25 minutes. 10  $\mu\text{L}$  of the appropriate agonist was added (Table 1) in assay buffer containing 1% DMSO (matched for 1% DMSO assay buffer). Kinetic measurements were then taken for 30 to 50 minutes. The response difference between the signal maximum and the last data point prior to agonist addition was used to generate  $\text{IC}_{50}$  plots using GraphPad Prism® software.

## How to DMSO Match Assays

In order to correctly DMSO match assays, first calculate the dilution factor of the compound to go in the source microplate, and then divide the stock compound concentration of DMSO by this factor. This gives the percentage of DMSO in the assay. Cells in the assay microplate can then be washed into an assay buffer containing the same percentage of DMSO.

## Example

- ▶ Compound stock is 10 mM in 100% DMSO
- ▶ Final concentration of agonist in the assay is 8  $\mu\text{M}$ ; the dilution is 1:1250
- ▶ Add 10  $\mu\text{L}$  of compound to a well containing 40  $\mu\text{L}$  of assay buffer. The compound is added to the source microplate 5X the final concentration required, resulting in a final dilution factor of 250X.
- ▶ 100% DMSO/250 = 0.4% DMSO
- ▶ To DMSO match, wash cells into an assay buffer containing 0.4% DMSO, hence compounds are added at the same DMSO concentration.

## Results

### Effect of DMSO Mismatch on $\text{IC}_{50}$

A cell-based assay was established to determine the tolerance of DMSO mismatches in compound addition. Cells were washed with buffer containing 1% DMSO (40  $\mu\text{L}$ ) and then 10  $\mu\text{L}$  of antagonist was added in serial dilution containing 0.5, 0.75, 1 (matched) or 2% DMSO, followed by agonist in matched conditions (1% DMSO). The delta post agonist addition was then plotted to generate dose-response curves. It was found that  $\text{IC}_{50}$  values were not significantly altered (in general within one log) (Table 2).

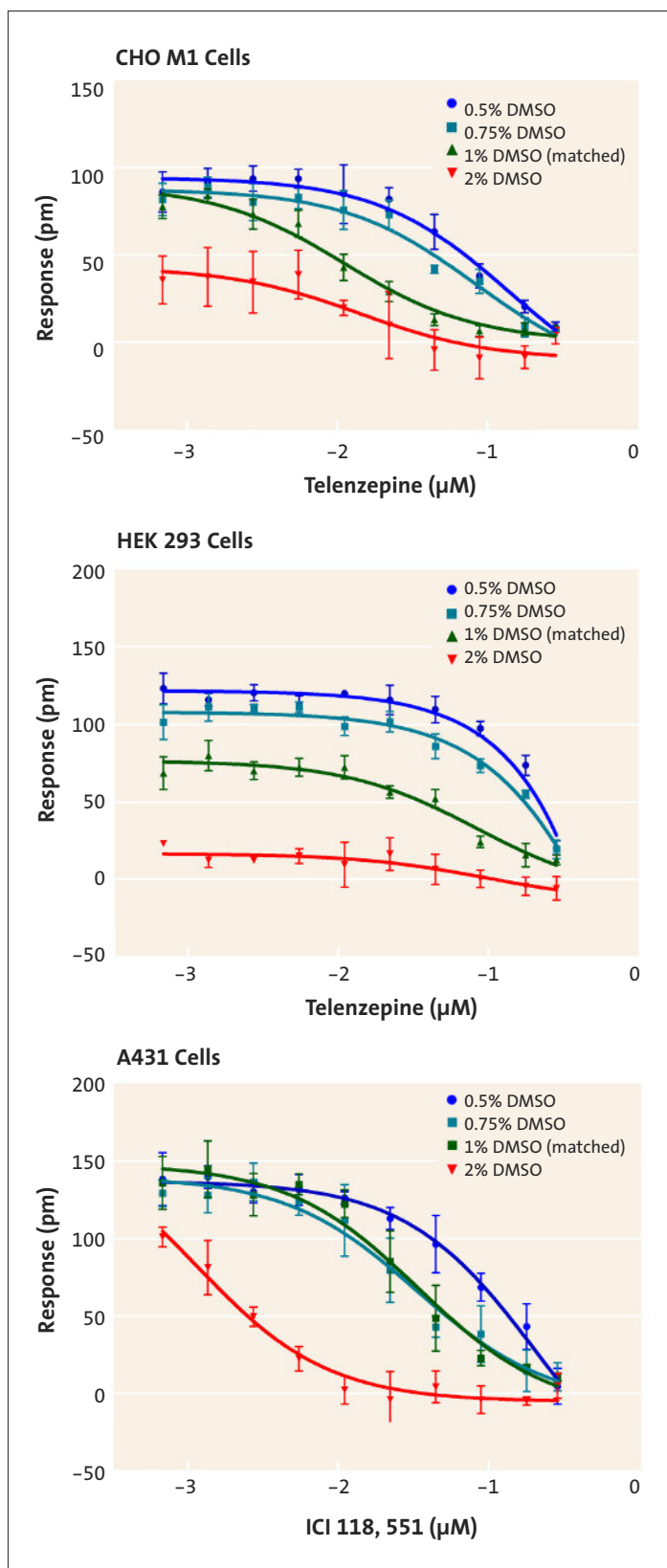


Figure 1. Dose-response Curves with Standard Antagonist in Mismatched Conditions in Three Different Cell Lines

### Effect of DMSO Mismatch on Signal Maximum

Experiments were performed to determine whether DMSO mismatching reduced the sensitivity of the assay by reducing the signal maximum. It was found that the magnitude of the signal is not greatly decreased with mismatches of 0.5 and 0.75%, however it is significantly decreased when 2% DMSO is added to wells containing 1% DMSO (Figure 1).

### Loss of Assay Sensitivity Due to DMSO Mismatch

The magnitude of the signal lost is dependent upon the cell line. The loss of assay sensitivity can be calculated by comparing the maximum signal obtained by adding agonist in a second addition relative to wells that received 2% DMSO in the first addition (mismatch) or 1% DMSO (matched). Table 3 shows the percentage of signal maximum relative to matched conditions and demonstrates that HEK 293 cells were particularly sensitive to mismatch conditions at 2% DMSO.

**Table 1.** Cell Line, Target Receptor and Compounds Used

Cell Line	Target Receptor	Antagonist	Agonist
A431	Gs	ICI 118,551 hydrochloride (Sigma, I127)	Epinephrine (Sigma, E1635)
CHO M1	Gq	Telenzepine (Sigma, T122)	Acetyl-b-methylcholine (Sigma, A2251)
HEK 293	Gq	Telenzepine (Sigma, T122)	Acetyl-b-methylcholine (Sigma, A2251)

**Table 2.** IC<sub>50</sub> Values (nM)\*

Cell Line	DMSO Added (%)			
	0.5	0.75	1	2
A431	207	33	34	1
CHO M1	135	81	11	14
HEK 293	Ambiguous	978	83	96

\*Values are generally within one log in the presence of DMSO Mismatch between 0.5 and 2%.

### Conclusions

- ▶ To maximize the sensitivity of an Epic® assay, one should buffer match as closely as possible.
- ▶ Quantities of DMSO added greater than a mismatch of 1% result in a loss of assay sensitivity, however the IC<sub>50</sub> values are not greatly affected.
- ▶ Sensitivity to DMSO mismatch is cell line dependent: in cases where DMSO mismatch is unavoidable it is recommended to determine the effect of the mismatch prior to scaling up the assay.

### Reference

1. Fang, Y., et al. Resonant waveguide grating biosensor for living cell sensing. *Biophys J* 91(5), 1925-40 (2006).

**Table 3.** Cell Line Specific Loss of Sensitivity with 2% DMSO Mismatch

Cell Line	Signal (%)
HEK 293	14
CHO M1	73
A431	95

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