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Epic[®]
system

Label-Free Cell-Based Screening for GPCR Agonists and Antagonists of the β_2 Adrenergic Receptor using the Corning[®] Epic[®] System

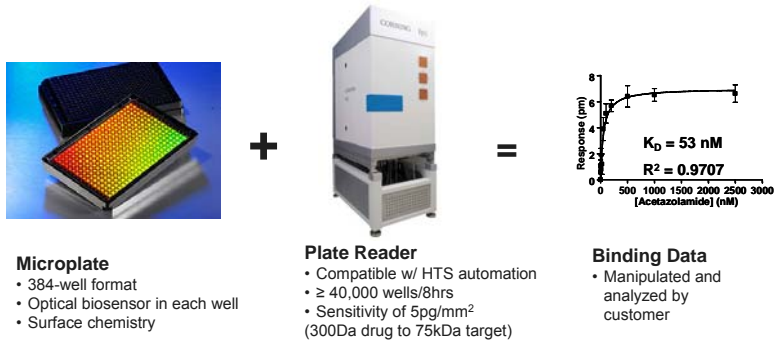
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Abstract

Heterotrimeric GTP-binding protein (G protein)-coupled receptors (GPCRs) constitute the largest class of drug targets currently under therapeutic investigation. Monitoring of the ligand-induced dynamic mass redistribution (DMR) in living cells upon receptor activation is accomplished using the Corning[®] Epic[®] System – a label-free microplate-based system incorporating resonant waveguide grating biosensors. The human epidermoid carcinoma cell line, A431, was used as a model system for screening the DMR response of an endogenously expressed GPCR. A431 cells, which express high levels of the beta2-adrenoceptor, were screened for known GPCR agonists and antagonists using the LOPAC 1280[™] compound library. The Epic System successfully identified all of the beta2-adrenoceptor agonists (7) and antagonists (1) in the library. To validate the results observed on the Epic System, A431 cells were also screened using a commercial cAMP technology. This study demonstrates the utility of the Epic System for cell-based high-throughput screening.

Corning® Epic® System

The Corning Epic System is a high-throughput, label-free detection platform that consists of SBS-standard 384-well microplates with optical sensors inside each well, an HTS-compatible microplate reader and a set of label-independent assay protocols. The Epic System is applicable to both biochemical and cell-based assays, and enables high-throughput screening of “intractable” targets.



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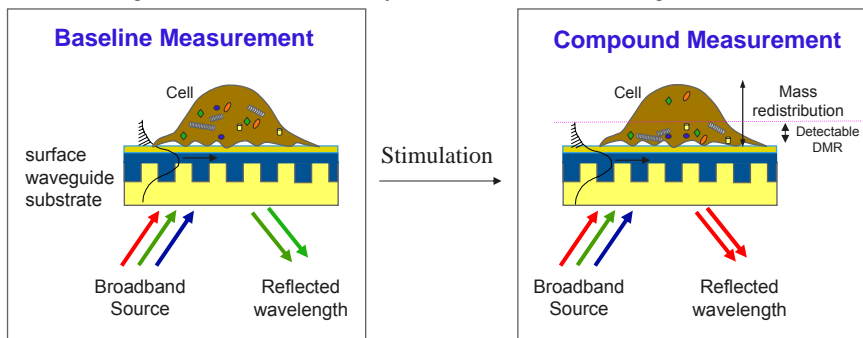
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Principle of Detection: Cell-based Assays on the Epic® System

- Measures changes in local index of refraction resulting from the ligand-induced dynamic mass redistribution (DMR) within the bottom region ($\sim 150\text{nm}$) of the cell monolayer.
- Change in index is manifested by a shift in resonant wavelength



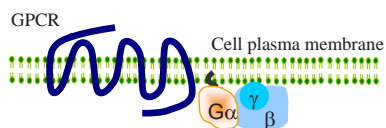
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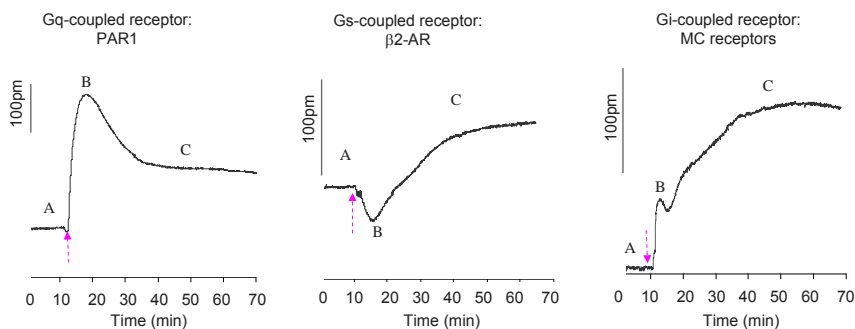
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Cell Assay Technologies with Epic® System Are Applicable to All Three Classes of GPCRs



Three classes of GPCRs, depending the G protein with which the receptor is coupled:

- Gαq – release of Ca²⁺
- Gαs – accumulation of cAMP
- Gαi – inhibiting cAMP generation



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Compound Screening on the Epic® System Using the LOPAC 1280™ Library

Cell Culture in Epic Microplates:

1. Seed A431 cells (20000 cells/well) and incubate overnight.
2. Serum starve for an additional 18-20 hours.
3. Incubate cells in assay buffer for 2 hours.

Epic System Procedure:

1. Take a baseline scan (~5 min).
2. Add LOPAC 1280™ compounds to the Epic microplate.
3. Incubate Epic microplate for 1 hour (Agonist screen).
4. Take a final scan (~5 min).
5. Add 10μL of epinephrine.
6. Incubate Epic microplate for 1 hour (Antagonist screen).
7. Take final scan (~5 min).

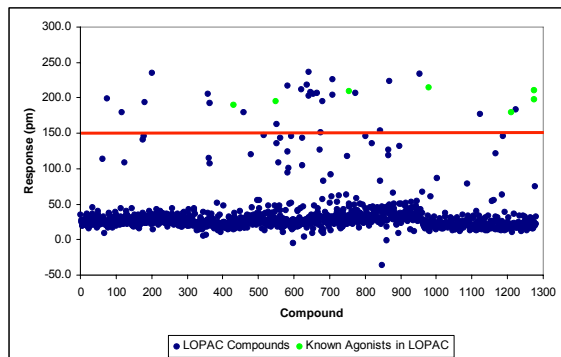
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Agonist Screen



Threshold set at 150µm

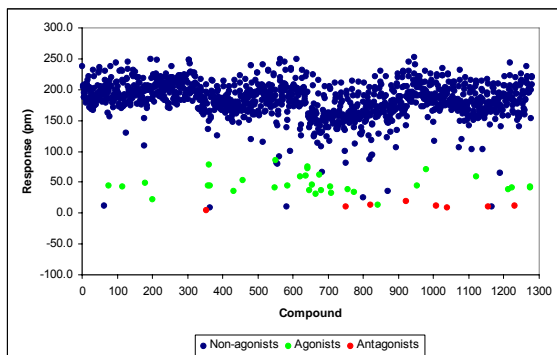
Total Hits 33 (2.6% of library)

Selectivity	Hits	% of Hits
Adrenergic	24	72.7%
Adenosine	3	9.1%
Dopamine	1	3.0%
Others	5	15.2%

No False Negatives

- Known agonists - LOPAC 1280™ library contains seven known β_2 AR agonists which were all identified as hits
- LOPAC compounds - all compounds with response >150µm are identified as hits

β_2 AR Antagonist Screen



Total Hits 8 (0.6% of library)

Selectivity	Hits	% of Hits
Adrenergic	5	62.5%
Others	3	37.5%

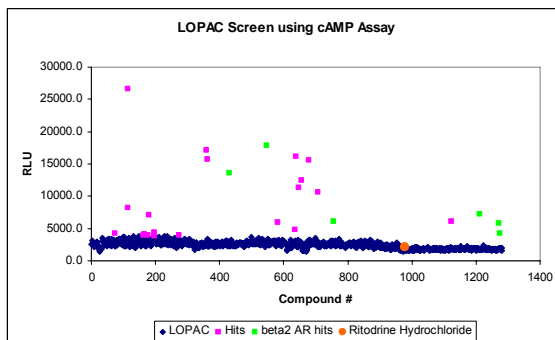
No False Negatives

- Antagonist - blocks epinephrine response in antagonist screen but non-responder in agonist screen
- Agonist - blocks epinephrine response in antagonist screen due to receptor desensitization in agonist screen
- Non-agonist - does not block epinephrine response in antagonist screen (exceptions are those compounds that block epinephrine response but had responses <150µm in agonist screen)

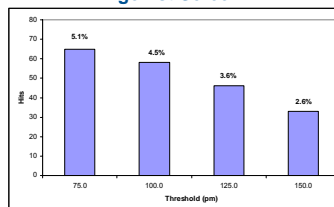
Comparison of Orthogonal Screening Technologies

- ▶ A leading cAMP assay was chosen as an orthogonal screening approach for comparison with the Epic® System.
- ▶ Applicable for cell-based screening of G-protein-coupled receptor (GPCR) activation.
- ▶ Uses Enzyme Fragmentation Complementation (EFC) technology to indirectly measure cAMP levels.
- ▶ A431 cells were screened with the entire LOPAC 1280™ library using a cAMP assay kit to identify G_{αs}-coupled agonists.

Agonist Screen



Threshold Analysis for Agonist Screen



Threshold set at 3837 RLU

Total Hits 26 (2.0% of library)

Selectivity	Hits	% of Hits
Adrenergic	18	69.2%
Adenosine	3	11.5%
Dopamine	2	7.7%
Other	3	11.5%

One false negative (Ritodrine hydrochloride)

Comparison of EC₅₀ Values

Epic® System

Epinephrine Dose-Response

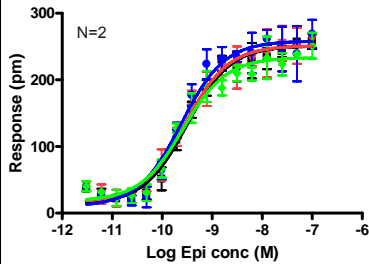


	Plate 1	Plate 2	Plate 3	Plate 4
EC ₅₀	0.31 nM	0.28 nM	0.24 nM	0.26 nM

cAMP Assay

Epinephrine Dose-Response

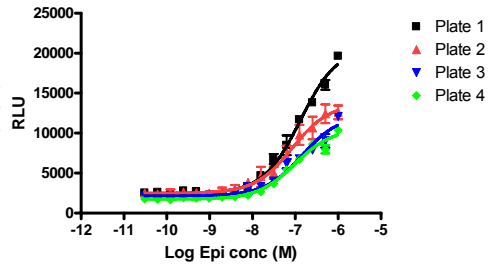


	Plate 1	Plate 2	Plate 3	Plate 4
EC ₅₀	131.4 nM	84.1 nM	143.1 nM	98.3 nM

- ▶ EC₅₀ values are consistent for all plates tested on each platform
- ▶ DMR measurement on Epic System produces EC₅₀ values 2-3 orders of magnitude lower than those obtained with the leading cAMP assay

Comparison of Z'

Epic® System

- Positive control:
▶ 1 μ M Epinephrine (N=16)
- Negative control:
▶ Buffer (N=16)

	Z'			
	Plate 1	Plate 2	Plate 3	Plate 4
	0.72	0.82	0.70	0.70

cAMP Assay

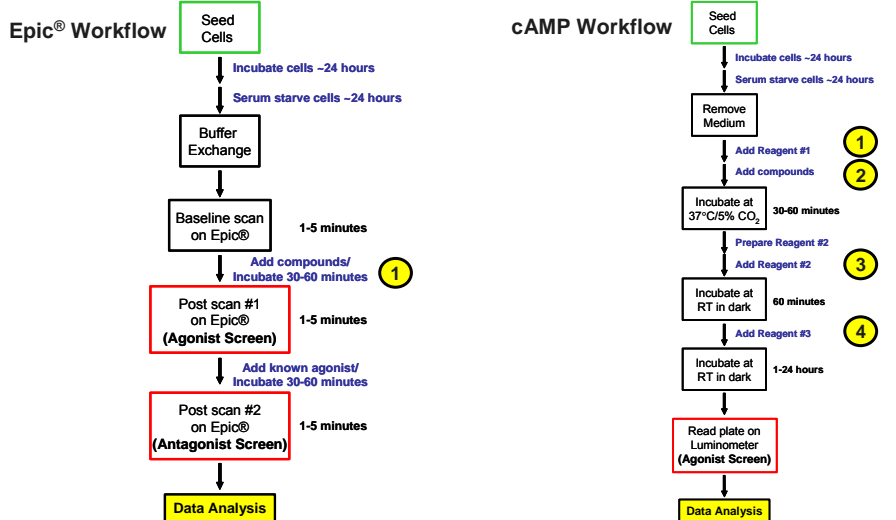
- Positive control:
▶ 1 μ M Epinephrine (N=14)
- Negative control:
▶ Buffer (N=14)

	Z'			
	Plate 1	Plate 2	Plate 3	Plate 4
	0.50	0.56	0.65	0.64

- ▶ Z' values >0.7 obtained for all 4 plates on Epic System demonstrating good assay robustness

Ease of Use in Screening

● = compound/reagent addition step



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Ease of Use in Screening

- ▶ Agonist and antagonist screens can be performed in the same experiment on Epic®.
 - ▶ cAMP assay cannot perform both agonist and antagonist screens in the same experiment.
- ▶ Agonist screens on Epic require only one compound addition step.
 - ▶ Agonist screens using cAMP assay require the addition of the compounds plus the addition of 3 different reagents.
 - ▶ No additional reagents need to be prepared on Epic. Only the compounds need to be prepared.
- ▶ Agonist screens on Epic require only one incubation step.
 - ▶ cAMP assay requires 3 incubation steps.
 - ▶ One incubation step in cell culture incubator
 - ▶ Two incubation steps in the dark
- ▶ Data can be collected immediately following compound addition on Epic.
 - ▶ Optimal data is acquired ~4 hours after compound addition using cAMP assay.

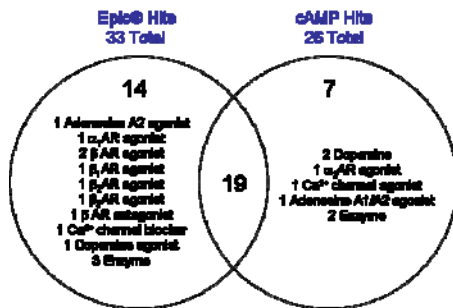
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Comparison of LOPAC 1280™ Screens



- ▶ All 7 known β_2 AR agonists present in library were successfully identified on the Epic® System
 - ▶ 6/7 known β_2 AR agonists were identified using cAMP assay resulting in one false negative.
- ▶ 19 compounds were identified on both platforms
 - ▶ 17 compounds were adrenergic agonists
 - ▶ 2 compounds were adenosine agonists.
- ▶ 14 additional hits were identified on Epic System
- ▶ Each of the 14 additional hits demonstrated receptor desensitization in the Epic antagonist screen, confirming that these compounds act through the β_2 AR pathway.