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Epic system

Label-Free Cell-Based Screening for GPCR Agonists and Antagonists Using the Corning® Epic® System

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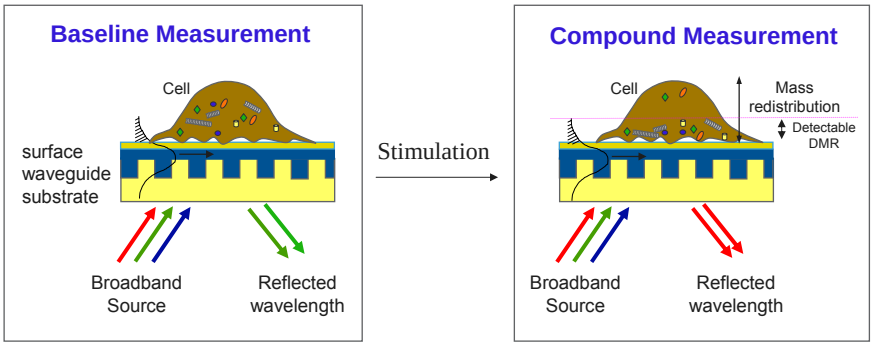
Abstract

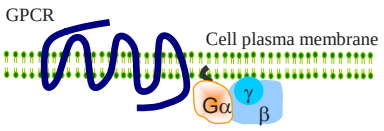
Heterotrimeric GTP-binding protein (G protein)-coupled receptors (GPCRs) constitute the largest class of drug targets currently under therapeutic investigation. Receptor stimulation by cognate ligands results in rapid translocation of intracellular components involved in signal propagation and cytoskeletal reorganization. Monitoring of the resultant dynamic mass redistribution (DMR) in living cells following receptor activation is accomplished using the Corning® Epic® System – a label-free and non-invasive 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 express high levels of the beta2-adrenoceptor, which is a prototypical member of the Gαs-coupled class of GPCRs. We first optimized assay conditions, including buffer composition and DMSO tolerance for a known beta2-adrenoceptor agonist, epinephrine. A431 cells were then screened for known GPCR agonists and antagonists using the commercially available LOPAC 1280™ compound library. The Epic System was able to successfully identify all of the beta2-adrenoceptor agonists (7) and antagonists (1) present in the library. This study demonstrates the utility of the Epic System for cell-based high-throughput screening.

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 (~150nm) of the cell monolayer.
- Change in index is manifested by a shift in resonant wavelength

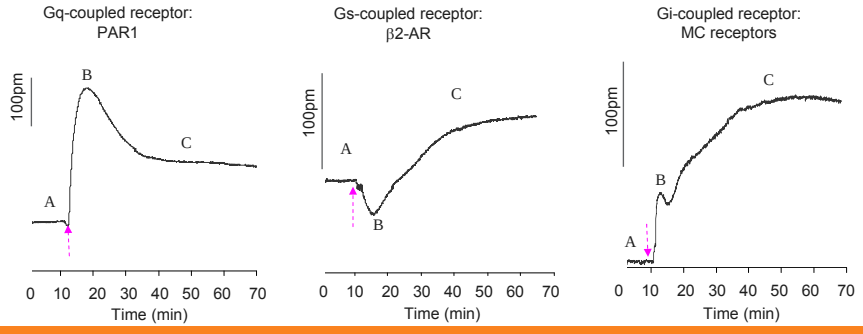


Cell Assay Technologies with Epic® System Are Applicable to All Three Classes of GPCRs



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



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

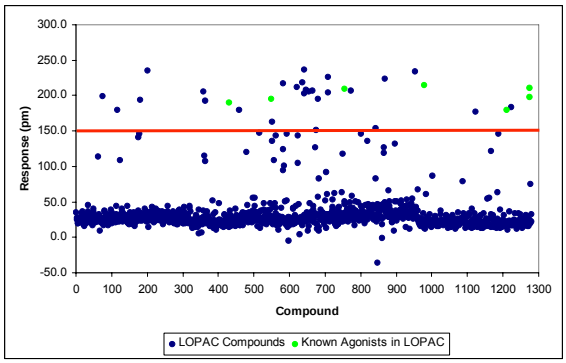
Cell Culture in Epic Microplates:

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).

Agonist Screen



Threshold set at 150pm

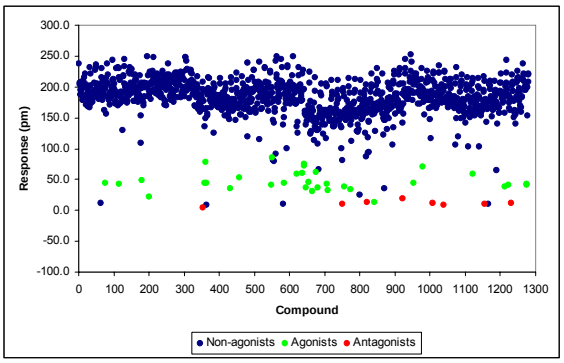
Total Hits **33 (2.6% of library)**

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_2AR agonists which were all identified as hits
- LOPAC compounds - all compounds with response >150pm are identified as hits

β_2AR 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 <150pm in agonist screen)

Comparison of Orthogonal Screening Technologies

- The 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 $G_{\alpha s}$-coupled agonists.

Ease of Use in Screening

Epic® System

Cell Culture:
- Use 10-20K cells/well
- Culture cells for 48 hr including serum starvation

Assay:
- Simple one-step addition of test compounds
- Data acquired directly after compound addition
- Data obtained in either HTS (end-point) or Continuous Scan (high content) format

cAMP Assay

Cell Culture:
- Use 5-10K cells/well
- Culture cells for 48 hr including serum starvation

Assay:
- Three steps of reagent or compound addition
- Incubation time of 30-60 min required after each add
- Additional liquid handling instrumentation required for large compound screen
- Optimal data acquisition at 4-5 hrs after compound add
- No Continuous Scan (high content) capability

Comparison of EC_{50} Values

Epic® System

Epinephrine Dose-Response

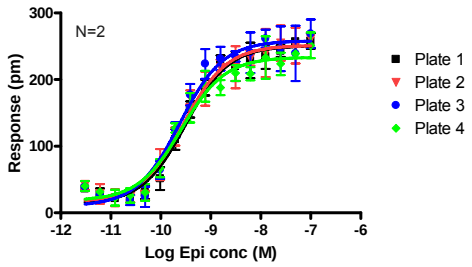


	Plate 1	Plate 2	Plate 3	Plate 4
EC_{50}	0.31 nM	0.28 nM	0.24 nM	0.26 nM

cAMP Assay

Epinephrine Dose-Response

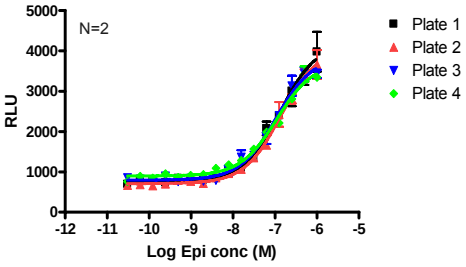


	Plate 1	Plate 2	Plate 3	Plate 4
EC_{50}	132.6 nM	137.1 nM	106.2 nM	109.9 nM

- EC_{50} values are consistent for all plates tested on each platform
- DMR measurement on Epic System produces EC_{50} values 2-3 orders of magnitude lower than those obtained with the leading cAMP assay

Comparison of Z'

Epic® System

Positive control:
- 1uM 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

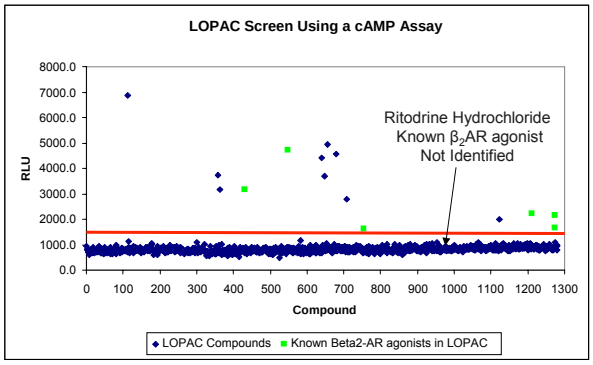
Positive control:
- 1uM Epinephrine (N=14)

Negative control:
- Buffer (N=14)

Z'			
Plate 1	Plate 2	Plate 3	Plate 4
0.66	0.31	0.53	0.64

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

Identification of Agonists Using a cAMP Assay



Threshold set at 1500 RLU

Total Hits **15 (1.2% of library)**

Selectivity	Hits	% of Hits
Adrenergic	14	93.3%
cAMP	1	6.7%

One False Negative

Comparison of LOPAC 1280™ Screens

- 6/7 known β_2AR agonists present in library were identified using a cAMP assay
  - All 7 were identified successfully on Epic® System
- 15 total hits were identified using the leading cAMP assay
  - 14/15 were adrenergic hits (1 other was cAMP)
  - All 14 adrenergic hits were also identified on Epic System
- 18 additional hits were identified on Epic System
  - 9 adrenergic agonists
  - 3 adenosine receptor agonists
  - 1 dopaminergic agonist
  - 1 calcium channel blocker
  - 4 non-receptor enzymes
- Each of the 18 additional hits demonstrated receptor desensitization in the Epic antagonist screen, confirming that these compounds act through the β_2AR pathway