
Application of the Corning[®] Epic[®] System to label-free functional GPCR assays

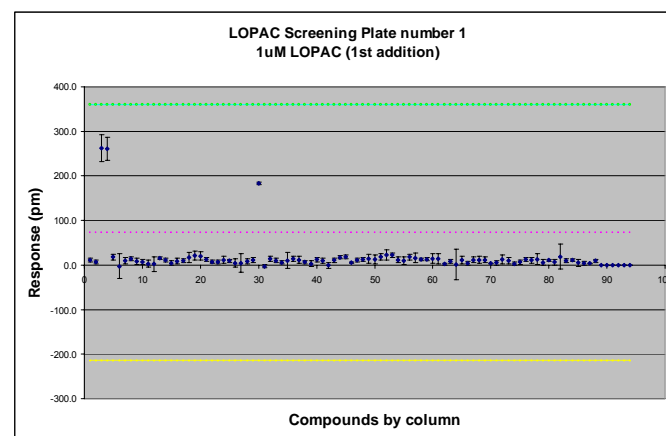
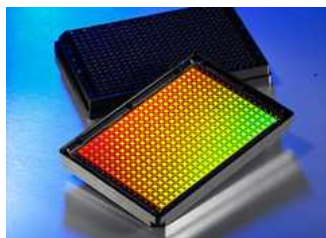
Kathy Dodgson
Lead Generation Biology
AstraZeneca R&D Charnwood

Overview

- Outline of the technology
- Application to all classes of G-protein
- Endogenous receptors
- Establishing a screening assay for a muscarinic receptor
- Summary

Corning® Epic® System

- A label-free drug screening system
 - An SBS-standard 384-well microplate with optical biosensors
 - An HTS-compatible microplate reader



Epic® Microplate

- 384-well format
- Optical biosensor in each well

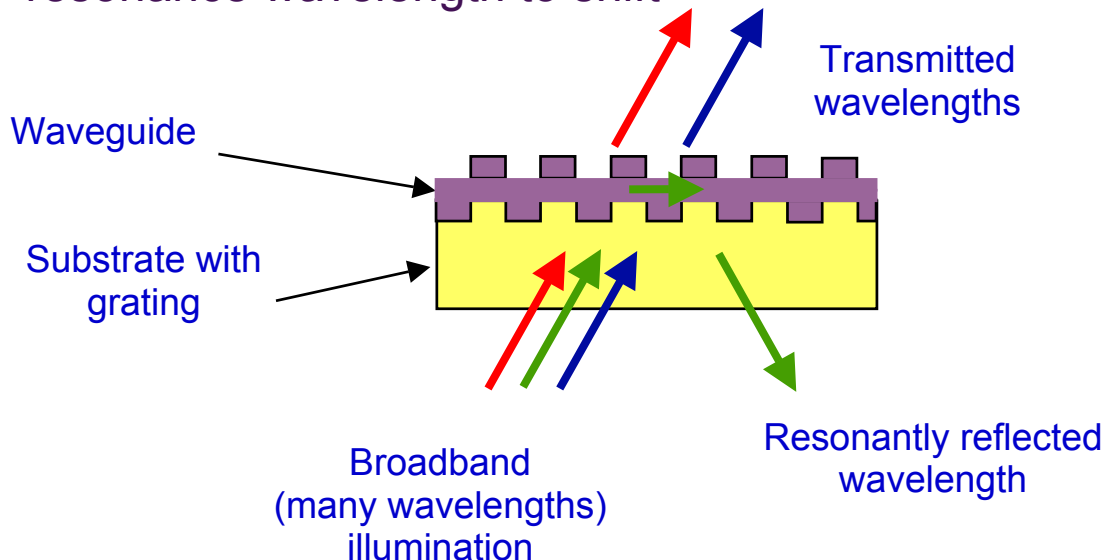
Epic® Microplate Reader

- Compatible w/ HTS automation
- $\geq 40,000$ wells/8hrs

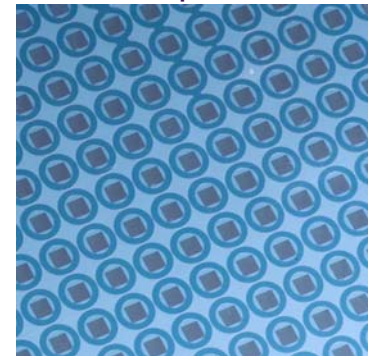
Assay Results

The Biosensor

- The substrate grating is covered with a thin dielectric layer; this forms the resonant waveguide grating.
- The sensor is illuminated with many wavelengths.
- Only a “single” wavelength that is resonant with the waveguide grating structure is strongly reflected.
- A change in the index of refraction at the sensor surface causes the resonance wavelength to shift

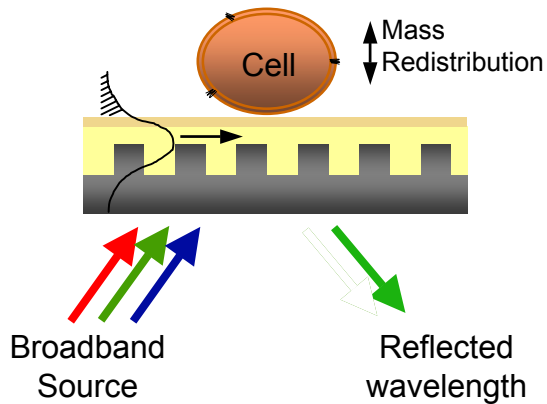


- The bottom of each well in the Epic® 384-well microplate contains a biosensor



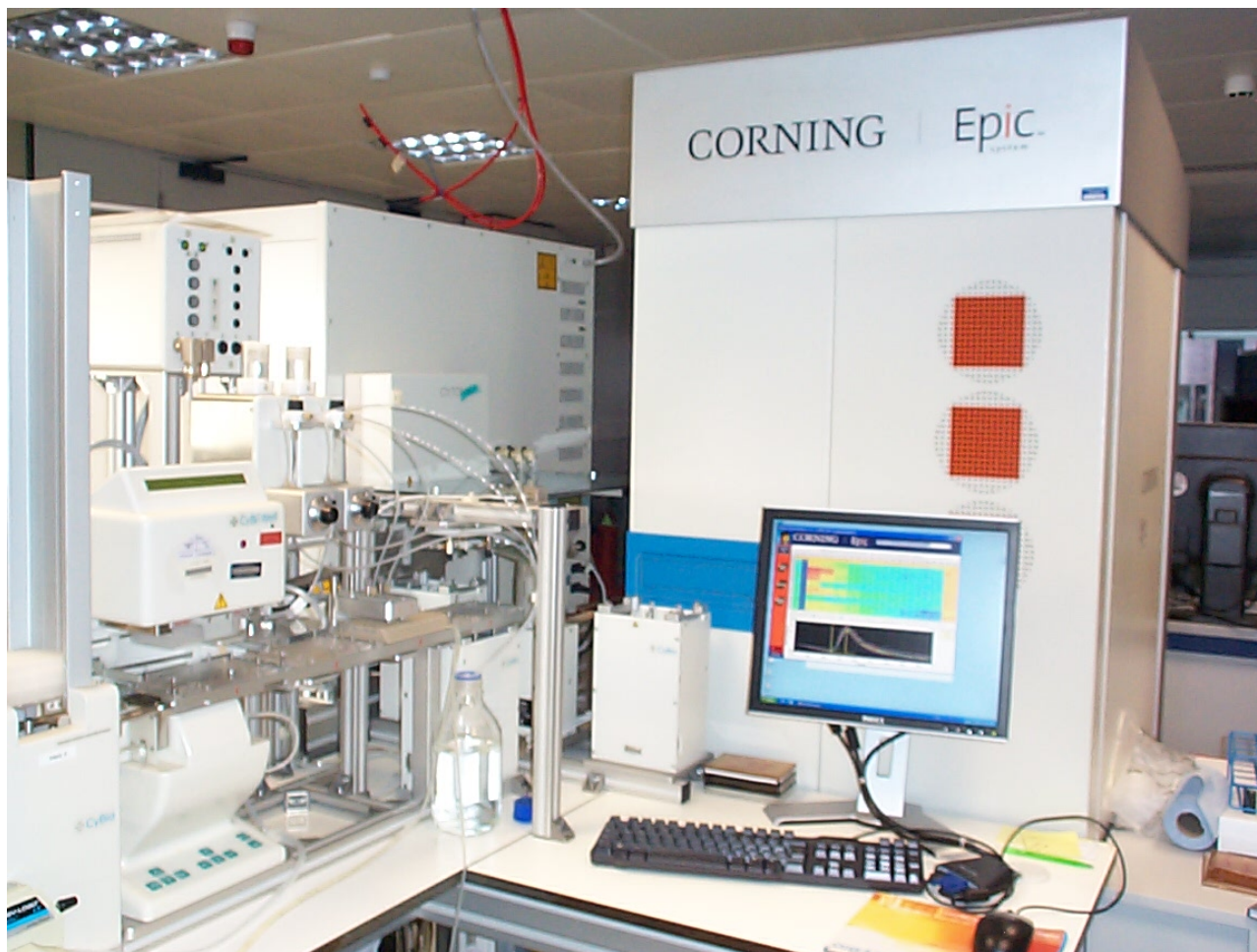
The Biosensor

Cell-Based Assays



- The Epic[®] System measures refractive index changes at the sensor surface
 - Light of the resonant wavelength couples into, and propagates down the waveguide.
 - A portion of the resonant wavelength extends into the first few hundred nanometers (~150 nm) above the surface of the waveguide, which defines the sensing region.
 - Cells can be plated onto the EPIC plate and their response to specific stimuli measured.
 - Dynamic mass redistribution within a cell causes index of refraction changes, resulting in a wavelength shift
- The Epic[®] System reports shifts, relative to an initial (baseline) measurement.

Integrated Epic[®] System

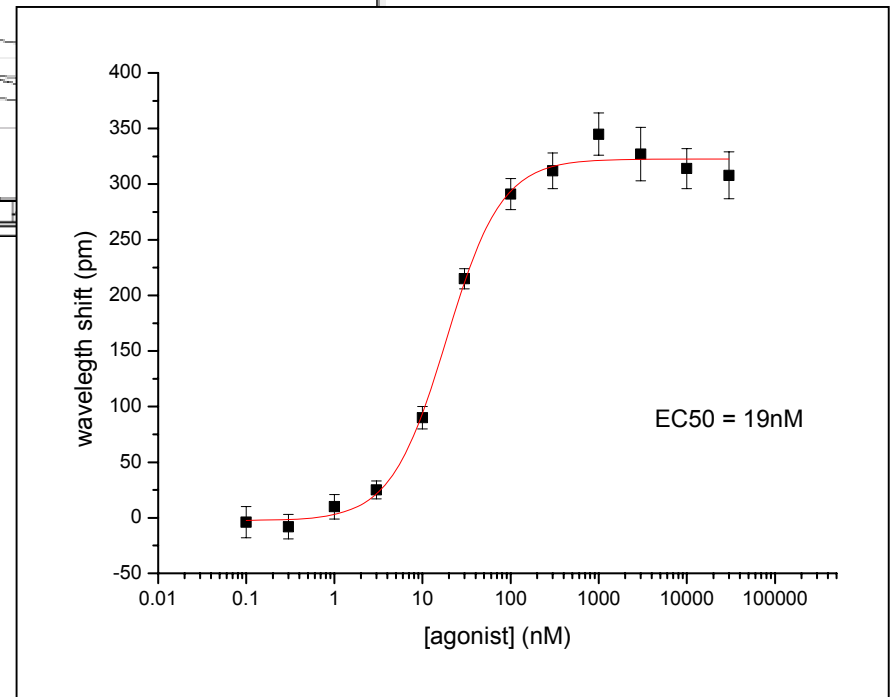
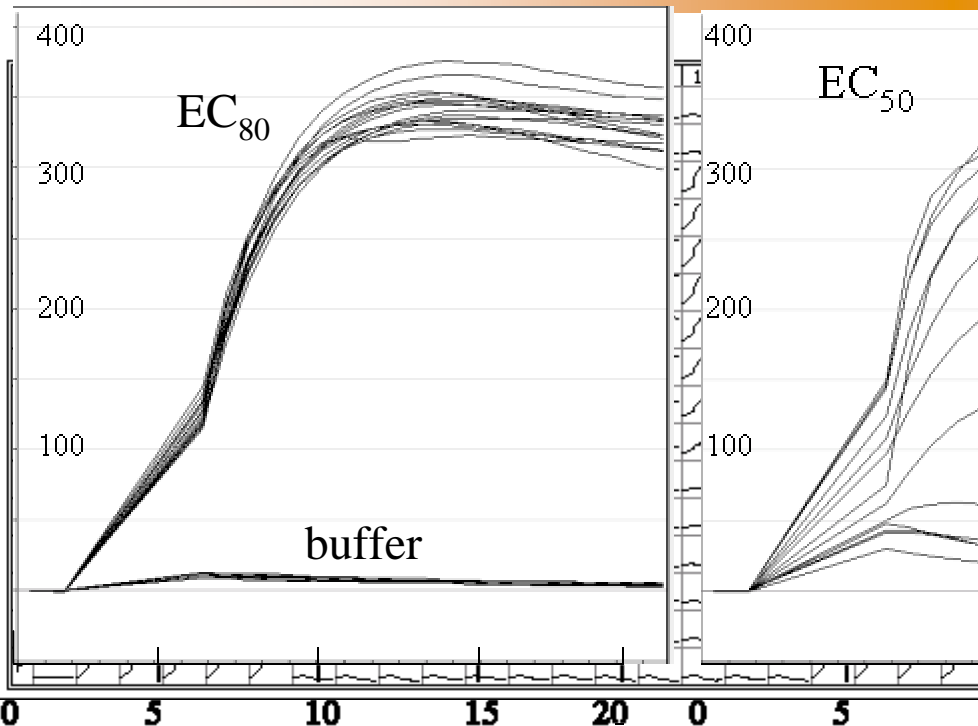


Cell-based assay examples

Assay Procedure

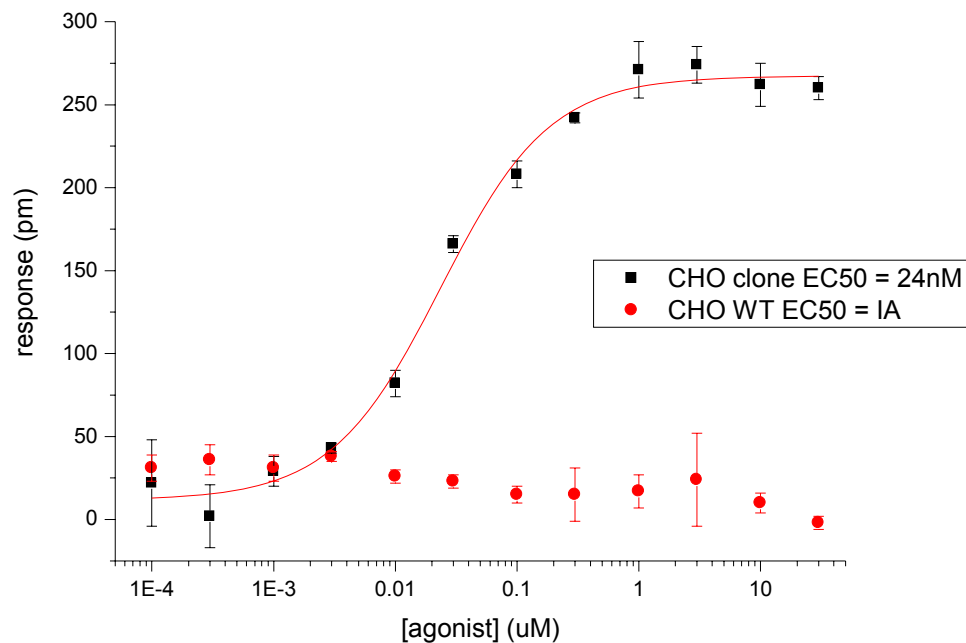
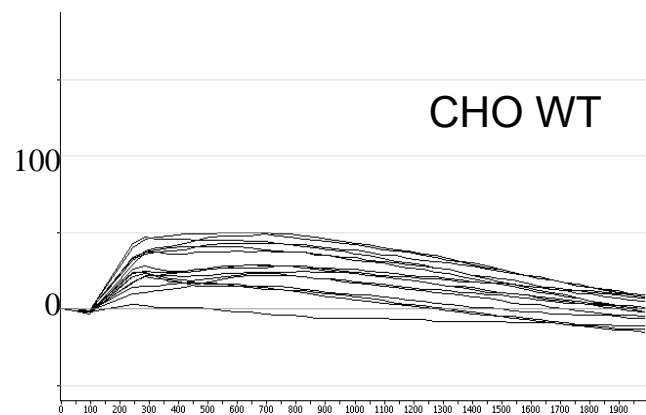
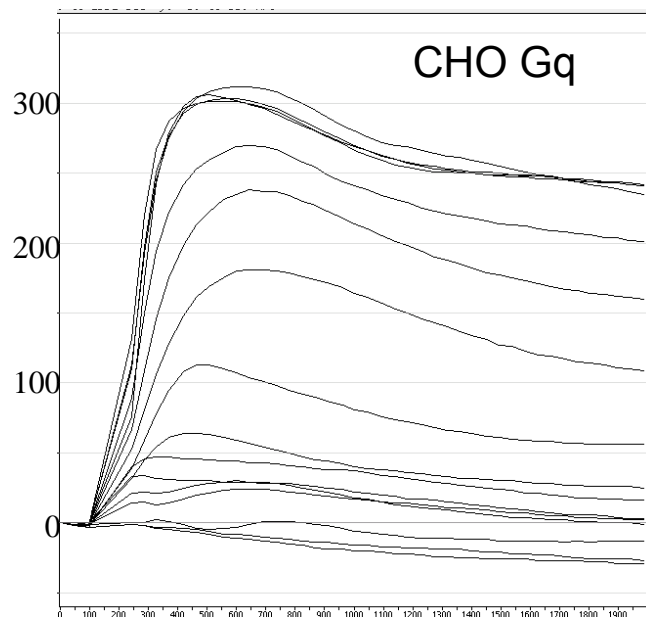
- Cells are plated onto the EPIC assay plate and allowed to adhere O/N
 - Uncoated standard plates for endogenous cells
 - Fibronectin coated plates for HEK and CHO clonal lines
- Prior to assay the cell media is aspirated (Tecan Power Washer) and replaced with assay buffer (HBSS, 20mM HEPES +/- 0.1%BSA)
- Compounds and agonists are prepared in the same buffer system.
- The plates are then equilibrated in the system incubator for 1hr.
- The EPIC reader measures an initial baseline
- The compounds are added to the assay plate using an integrated CyBiWell pipettor
- The agonist is added and further scans are made

Example of a Gq Receptor



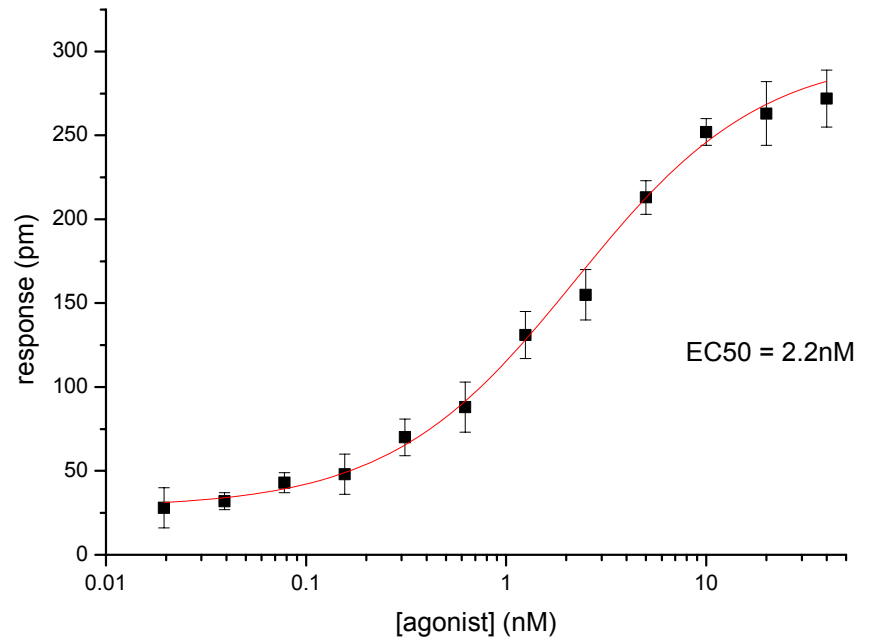
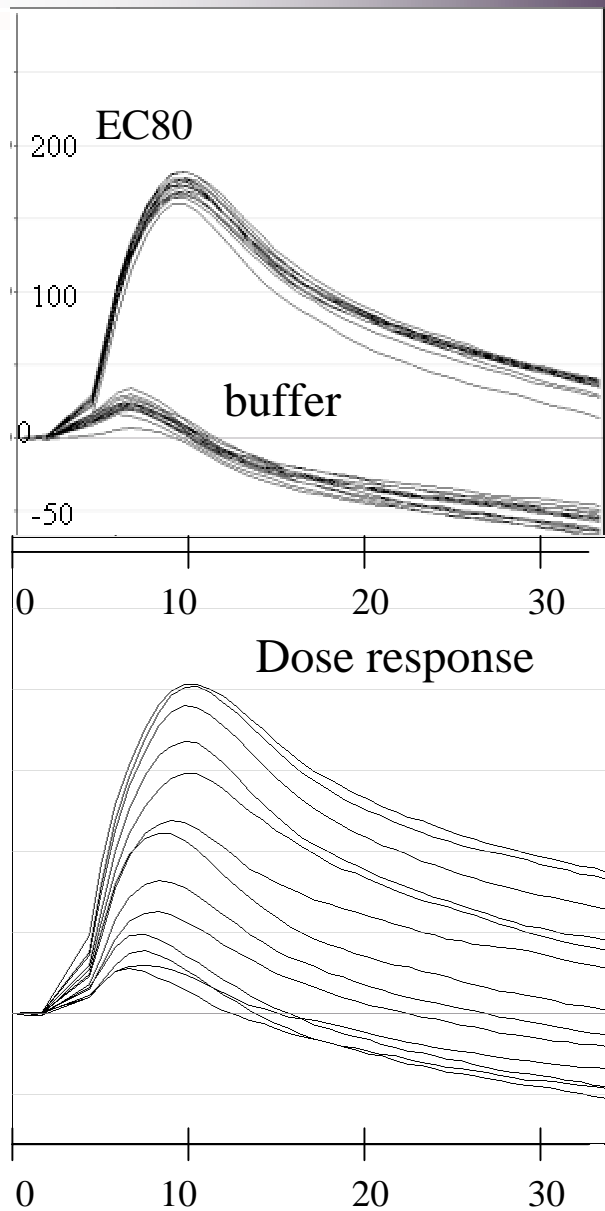
	Agonist	buffer
mean	308	-8
stdev	20	10
Z'	0.72	

CHO WT comparison



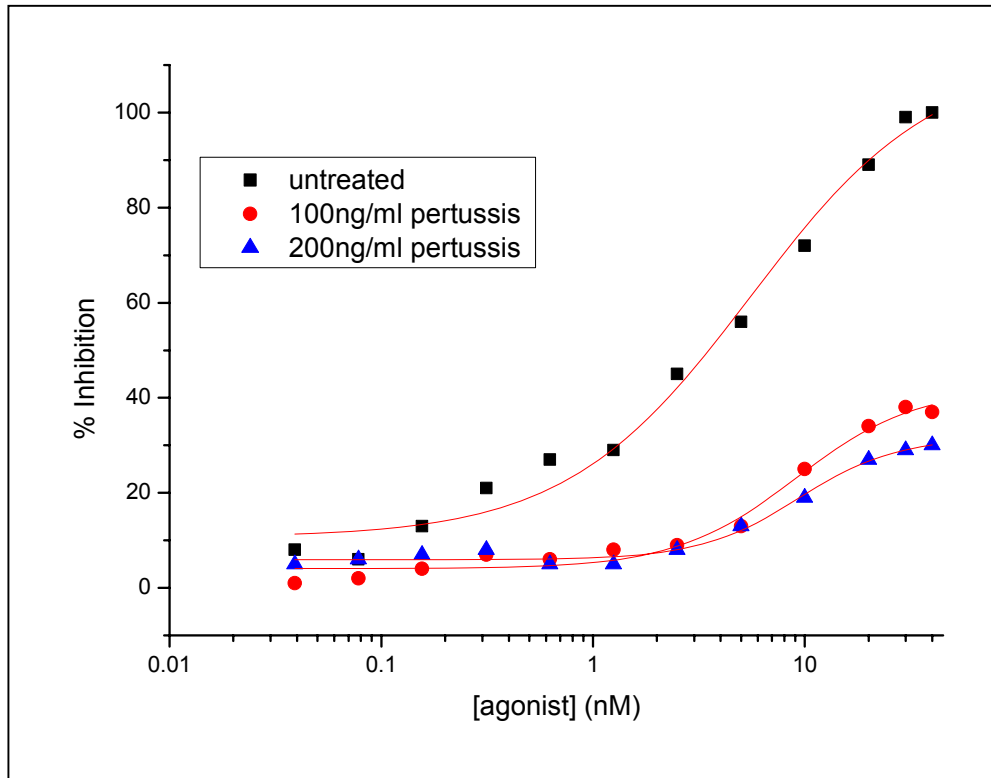
- CHO WT cells did not generate a response to the agonist.
- Both cell types responded to ATP (not shown)

Example of a Gi Receptor



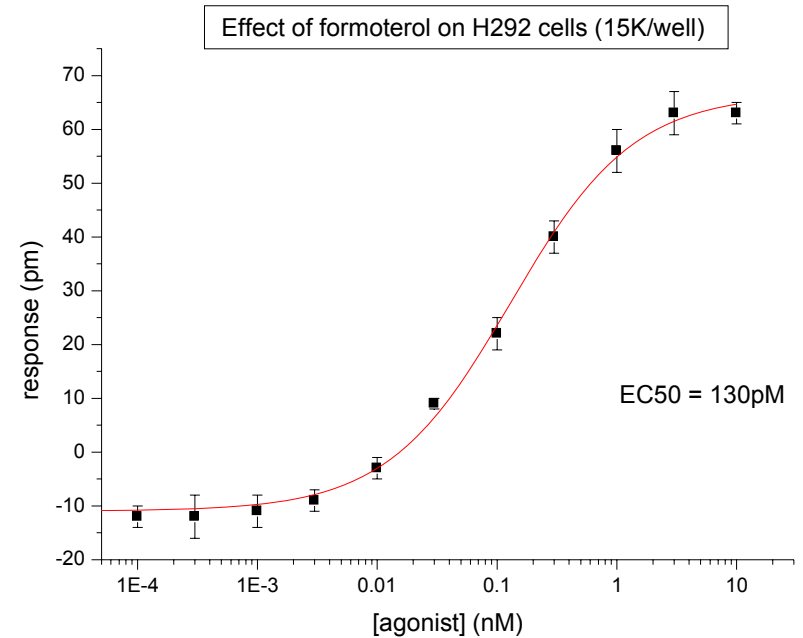
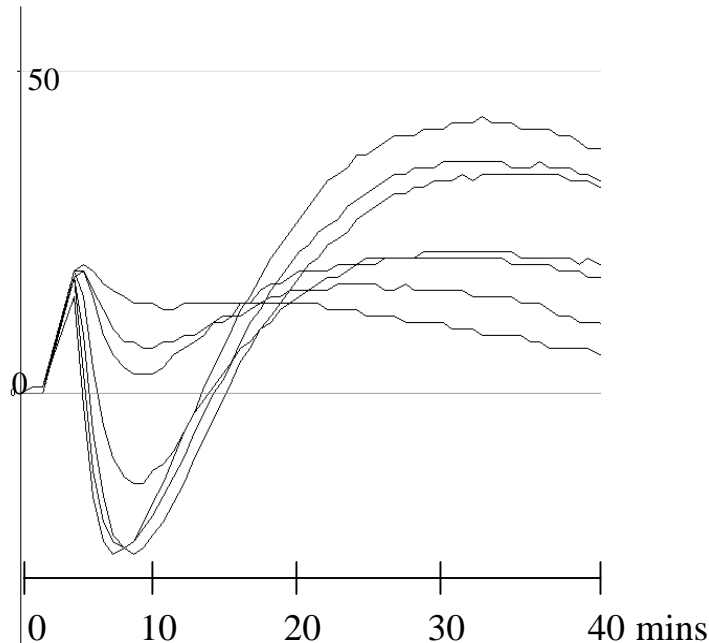
- HEK cell line
- Chemokine receptor
- EC₅₀ similar to other assay formats

Effect of Pertussis on Gi response



- Response decreased by pre-treatment with pertussis toxin
- 200 ng/ml PTX decreased the response by 70%

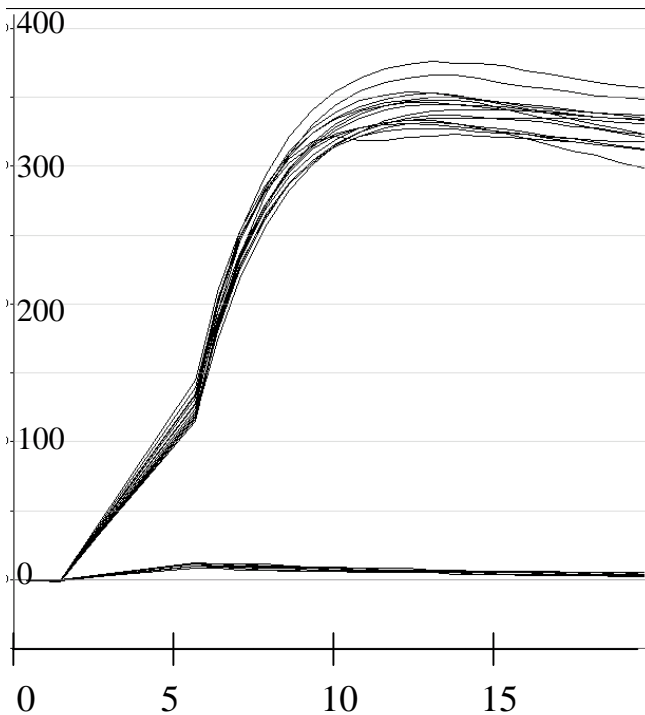
Example of a Gs Receptor



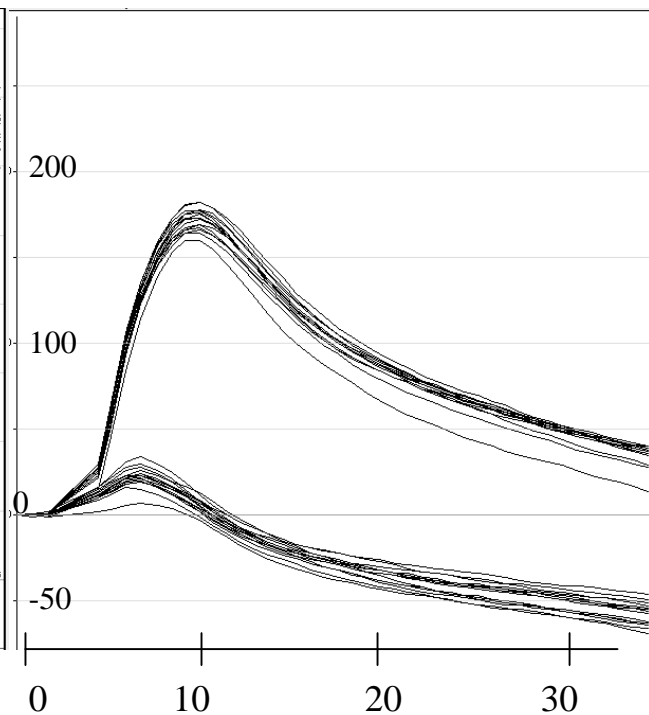
- H292 is an epithelial cell line
- Cells adhere to uncoated plate
- Endogenous Gs response for beta2 receptor

Summary of GPCR Responses

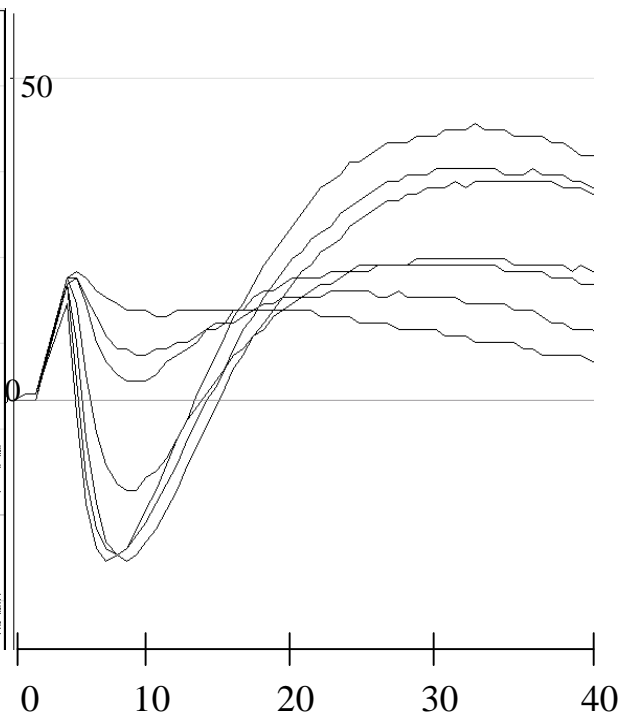
Gq response



Gi response



Gs response



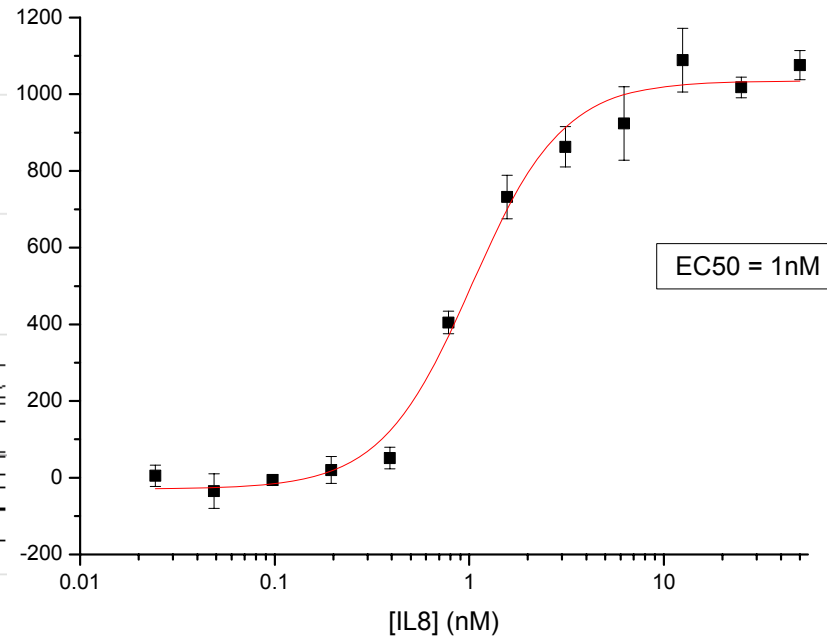
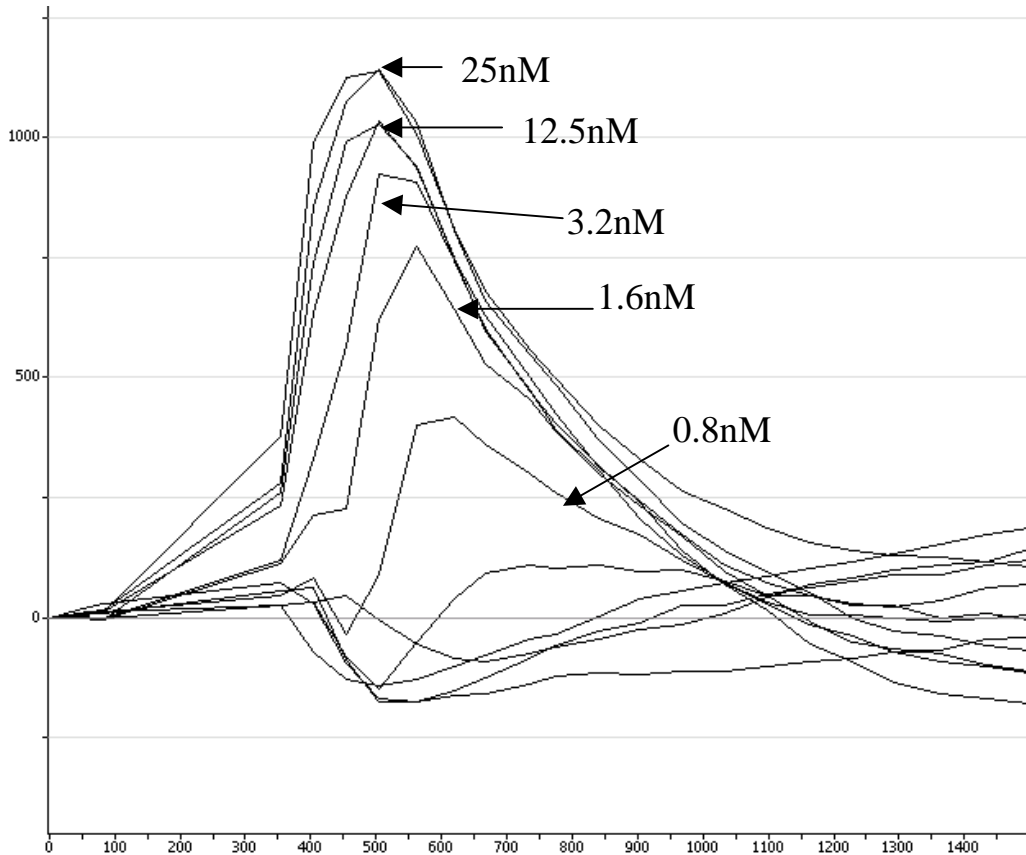
- The EPIC[®] is able to measure distinct responses for the 3 types of GPCR

Endogenous Receptors

Human Neutrophils

- Human neutrophils were prepared by density gradient centrifugation
- They were resuspended in HBSS/HEPES plus 0.1% BSA and plated onto uncoated EPIC assay plates at 80 to 50K/well
- The plates were centrifuged briefly
- Agonists were prepared in the same buffer system
- The effect of IL8 and GRO α was measured using the Epic[®] System

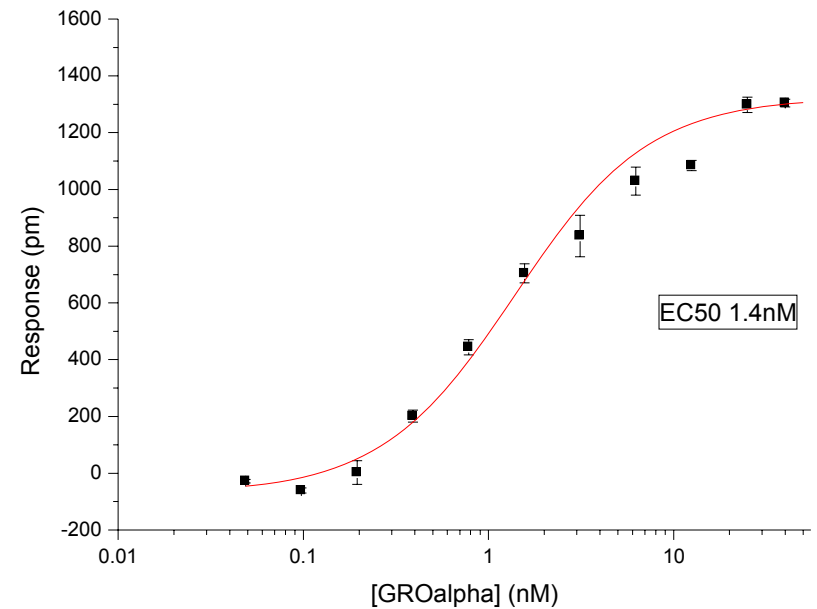
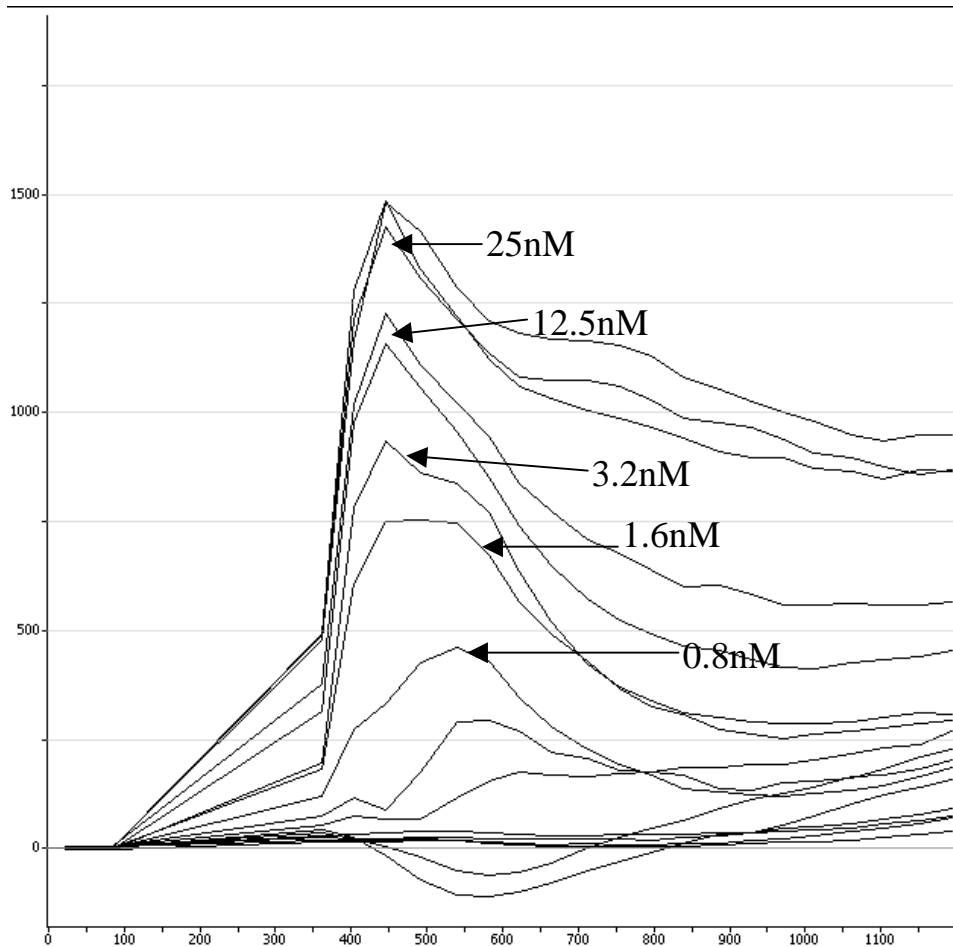
Neutrophil Response to IL8



60K neutrophils/well

FLIPR EC₅₀ = 1nM

Neutrophil Response to GRO α



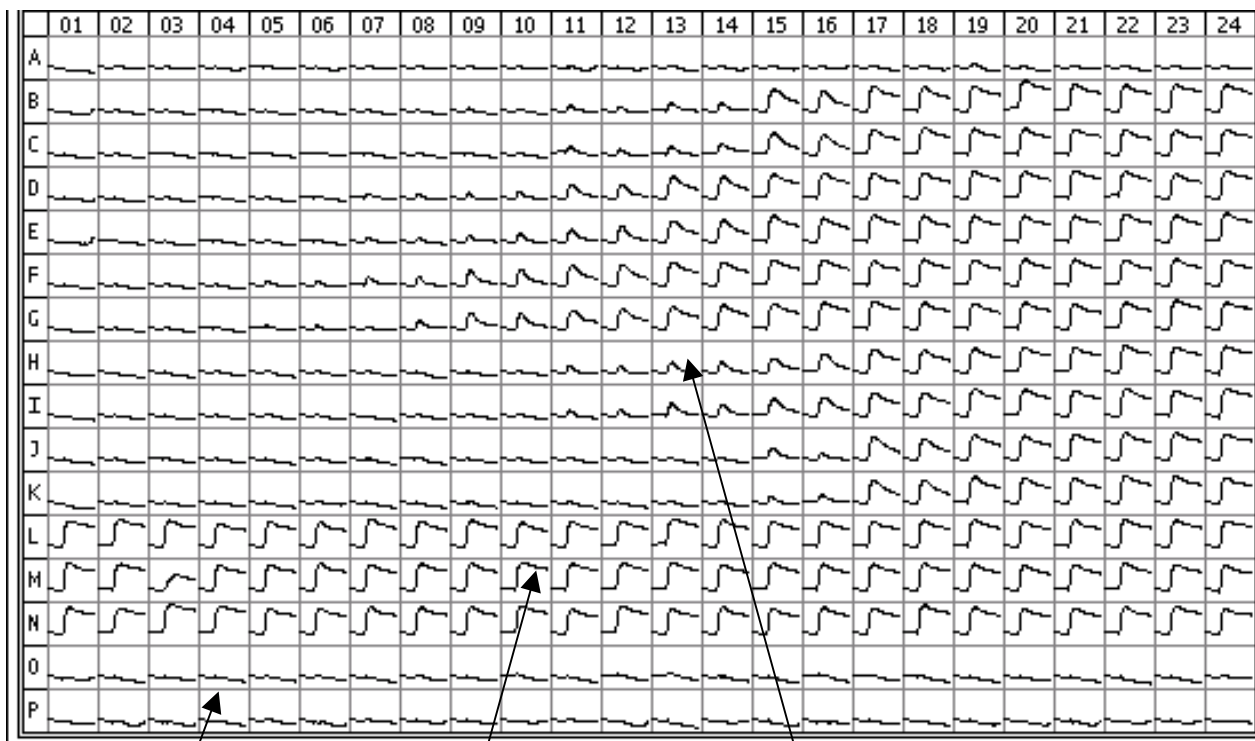
60K neutrophils/well

FLIPR EC₅₀ = 0.5nM

Screening Assay

Muscarinic Antagonist Screening

↓ Antagonist →



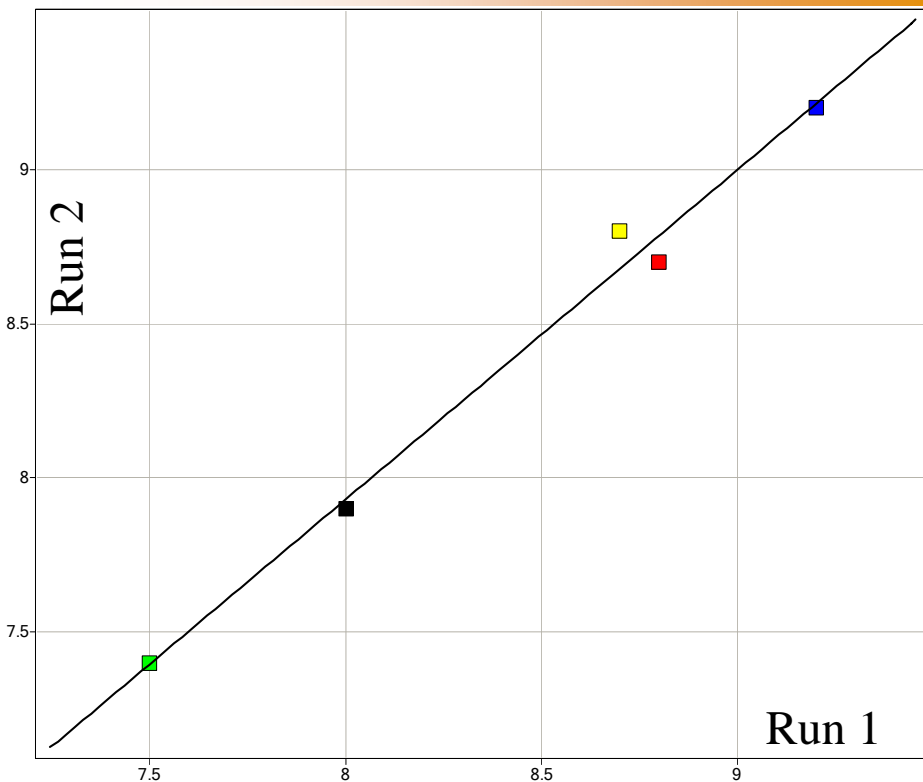
- Cells plated out O/N
- Cells washed and assay buffer added
- Initial baseline read
- Compounds added to adherent cells
- 0.25% DMSO final
- Agonist then added (0.25% DMSO)

Buffer controls
(O - P)

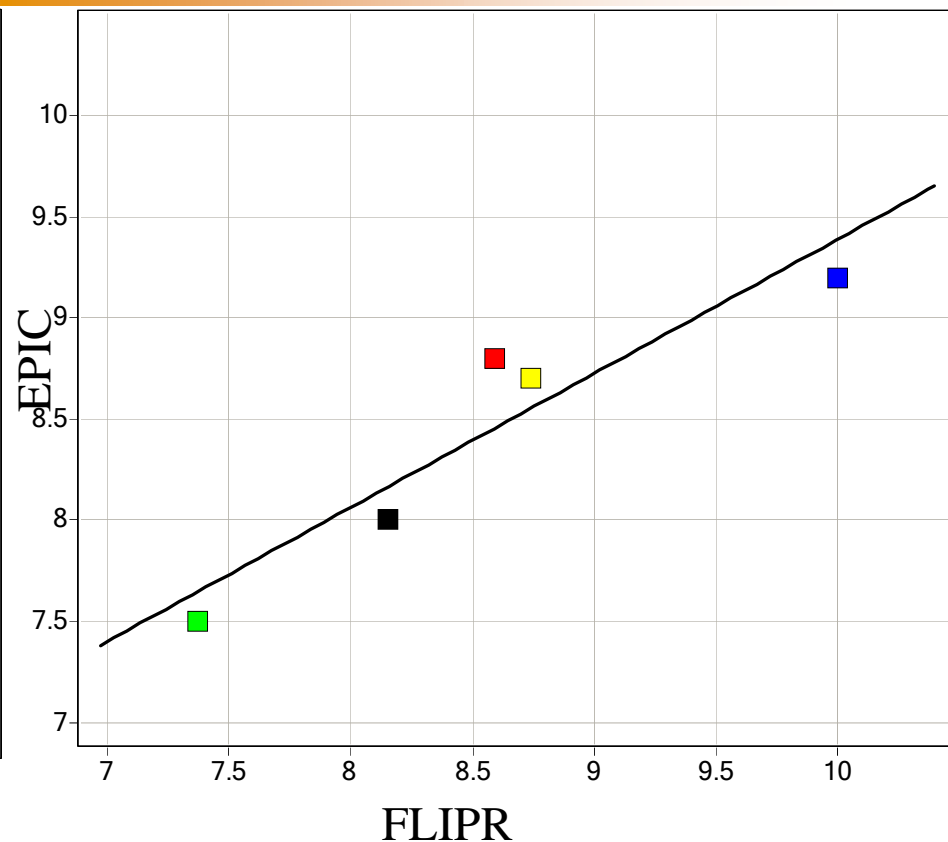
EC80 control
(L - N)

Antagonist
(B - K)

CHO antagonist rank order

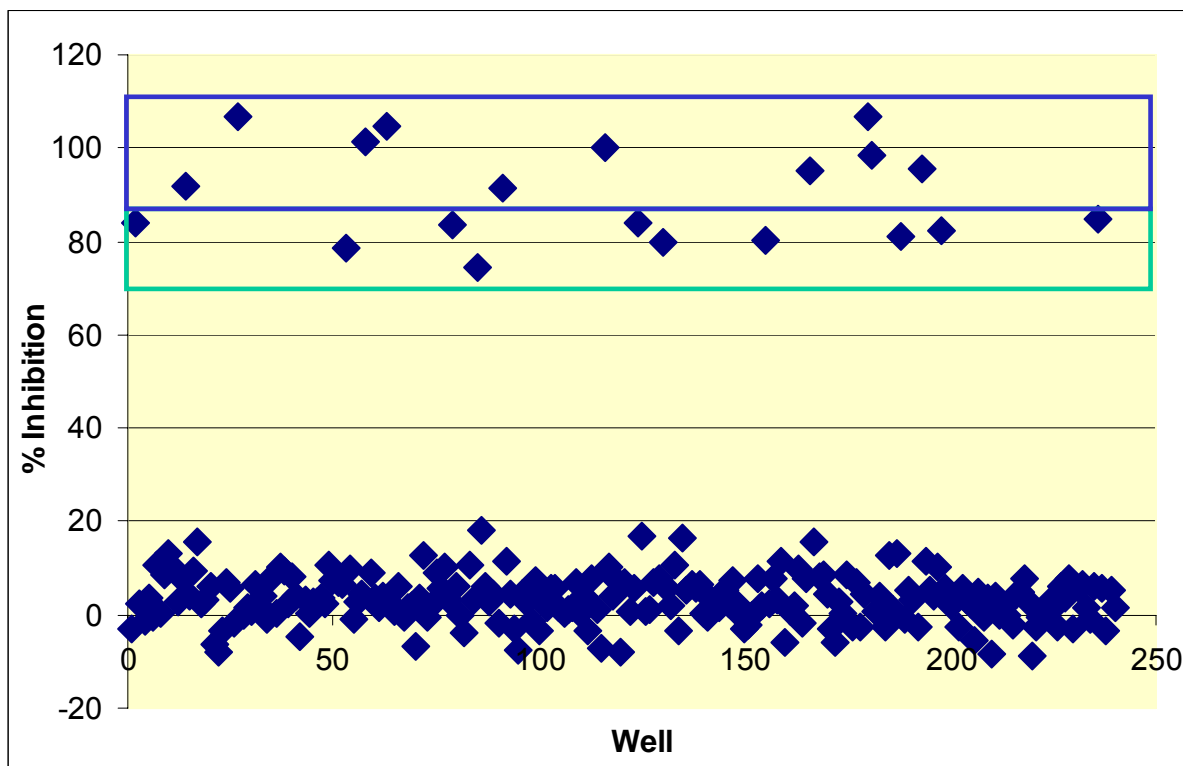


- Five standard compounds were tested on two days
- Equivalent pIC50 values were obtained



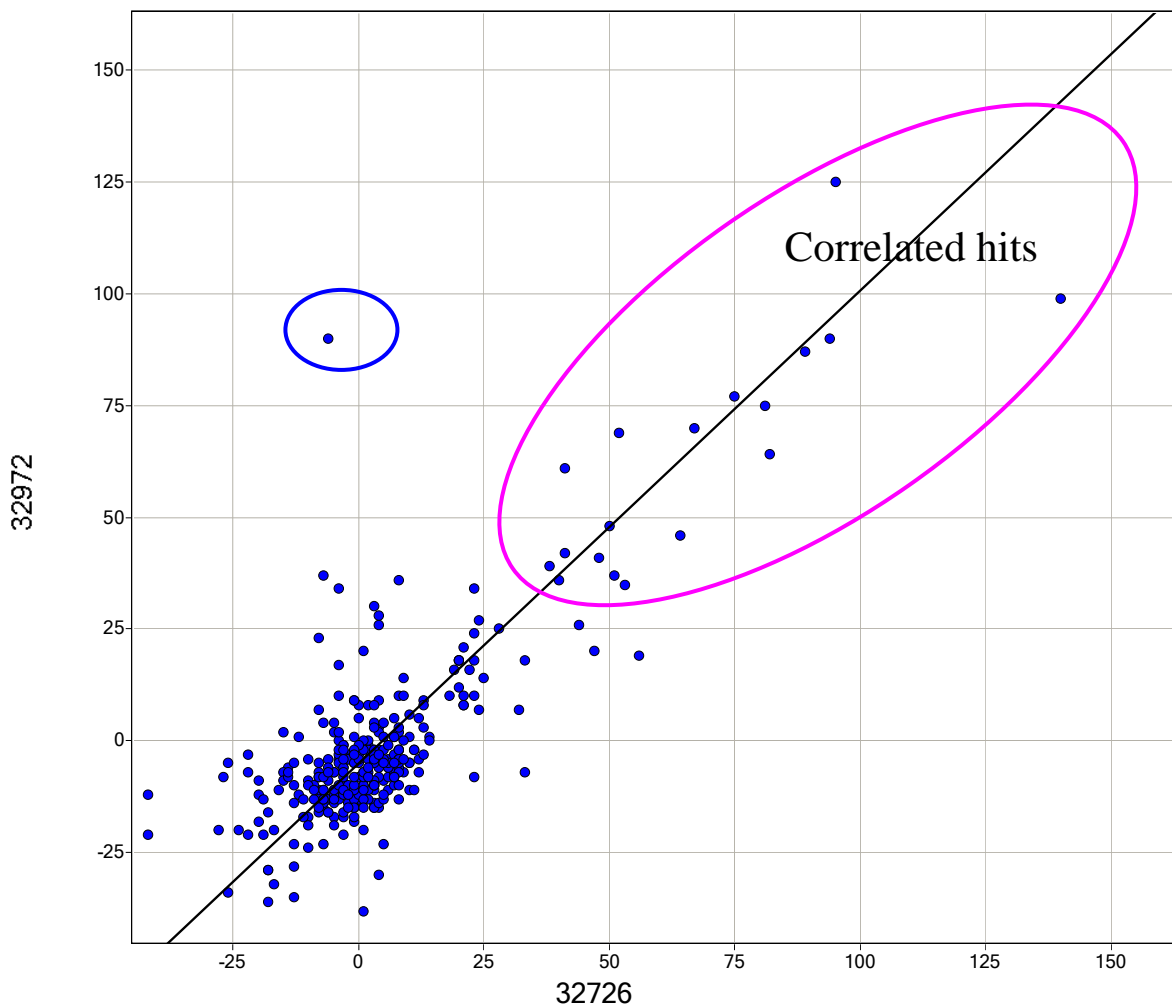
- The pIC50 values correlated well with FLIPR data

CHO Gq Screening Test



- 20 wells were spiked with a standard compound at 2 concentrations
- All the spiked wells were identified
- No false positives >20%
- The Z' value was >0.6

Correlation of Hits



- A compound plate containing 320 compounds was tested in duplicate on the same day
- Line of correlation $R^2 = 0.81$

Summary

- We have shown that the Epic[®] System can measure specific functional responses to cloned Gi, Gs and Gq GPCRs, with distinct temporal relationships.
- The technology is also sensitive enough to measure endogenous responses offering the possibility of performing assays in a biologically relevant system
- We are using the Epic[®] System for orthogonal screening post HTS. We intend to screen the first HTS on the system next year.
- We have also employed the EPIC[®] for compound/protein binding assays (See Philip Rawlins' poster).

Acknowledgements

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- Philip Rawlins
- Martin Coldwell
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CORNING

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