



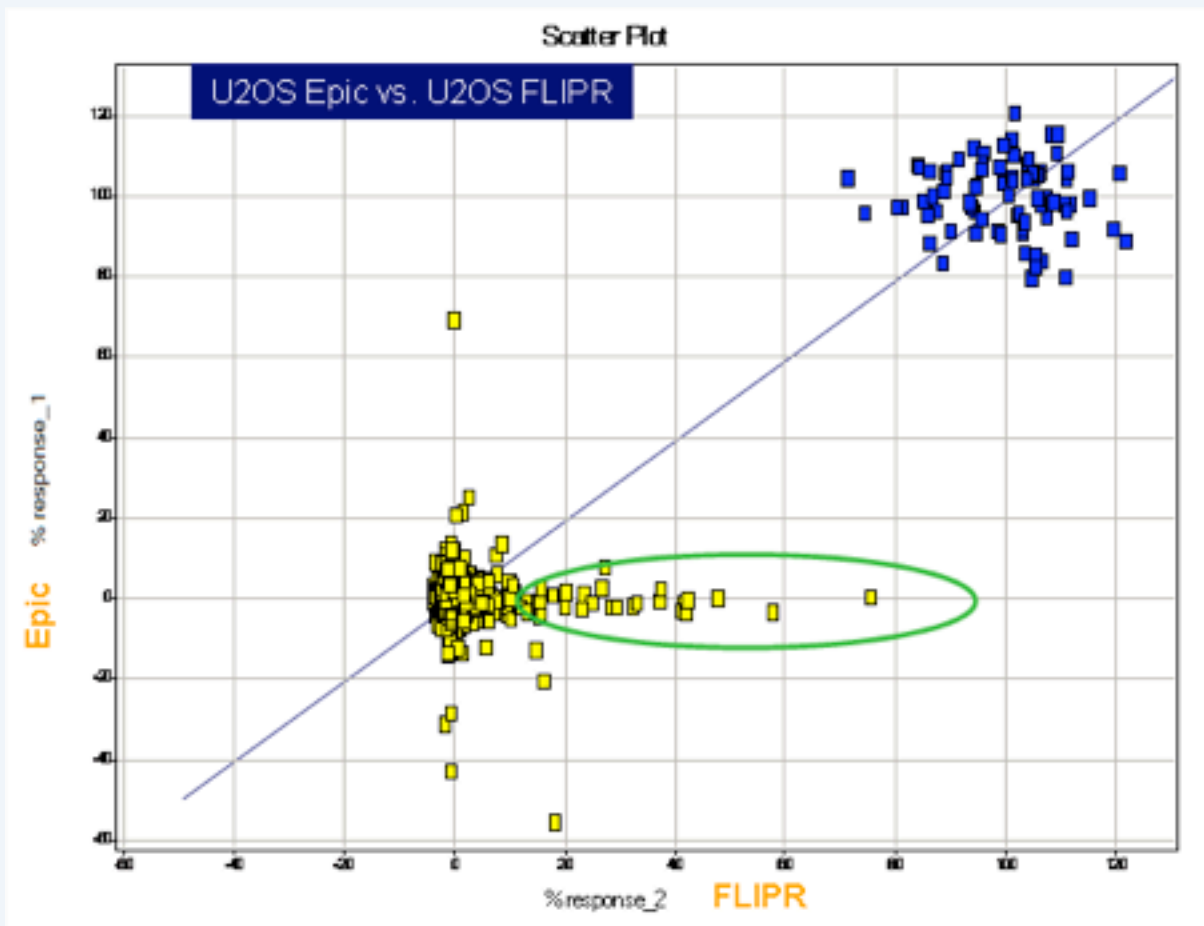
GlaxoSmithKline

# **Epic<sup>®</sup> System Evaluation**

## **May/June 2007**

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Steve Rees (SCP Harlow)  
Magalie Rocheville (BR&AD, GSK)  
Alan Wise (SCP Harlow)

# Epic® (15' end-point) vs. FLIPR U2OS robustness

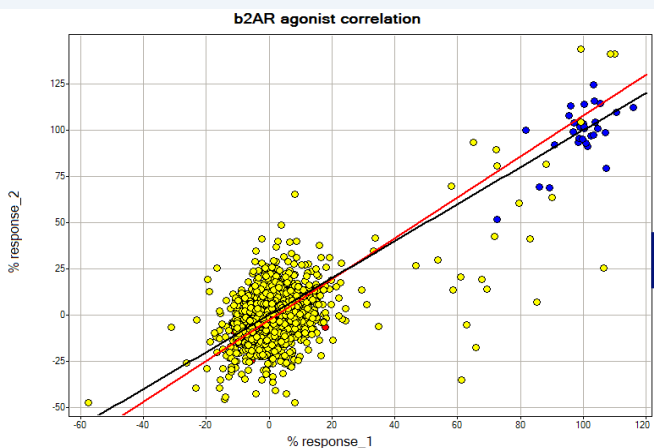


Replicate	Samples	No. Hits	Cut-Off	HR (%)
Epic (1)	1408	11	11	0.8%
FLIPR (2)		93	3	6.6%

# β2 AR assay Robustness

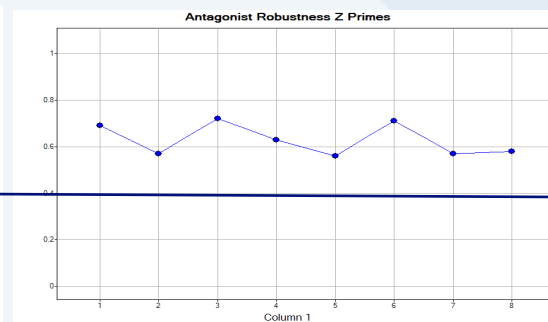
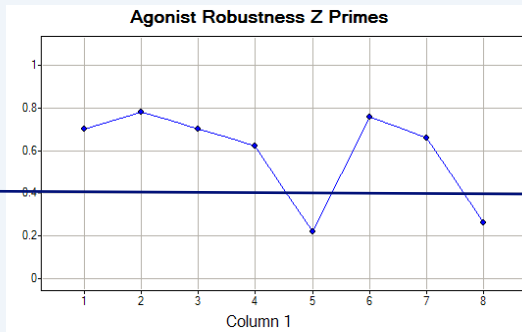
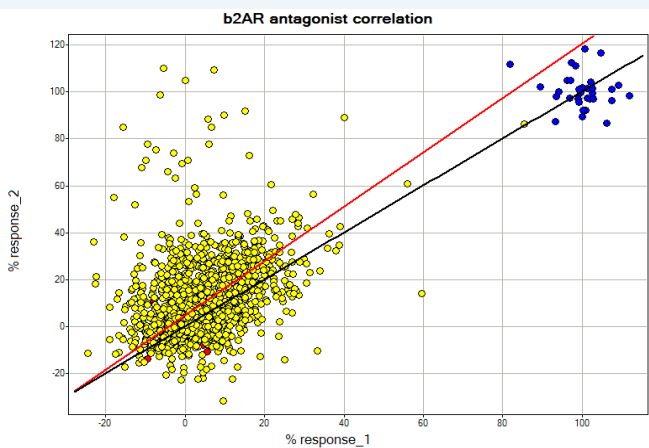
ROBUSTNESS n=2, 5uM 1% DMSO

## AGONIST MODE



■ 100% activation/inhibition  
■ 0% activation/inhibition

## ANTAGONIST MODE



Replicate	# Samples	Rob Cut-off	N hits	HR(%)
b2AR Epic Set1	1408	22	36	2.6
b2AR Epic Set2		34	24	1.7

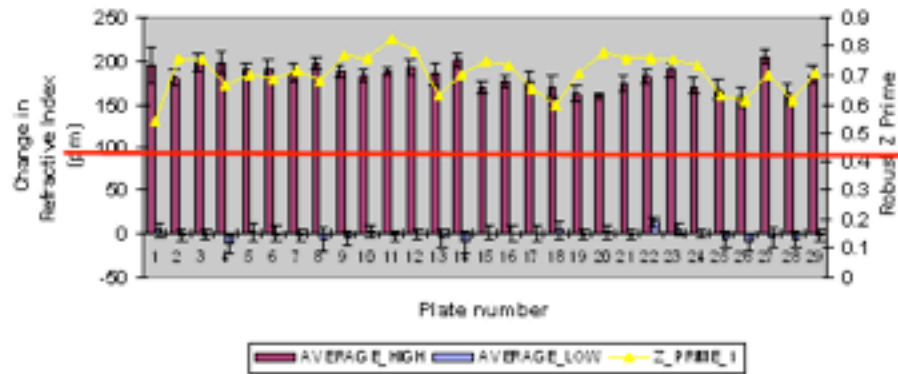
Replicate	# Samples	Rob Cut-off	N hits	HR(%)
b2AR Antagonist Set 1	1408	31	13	0.92
b2AE Antagonist Set 2		52	33	2.34

(False positives due to fungal infection)

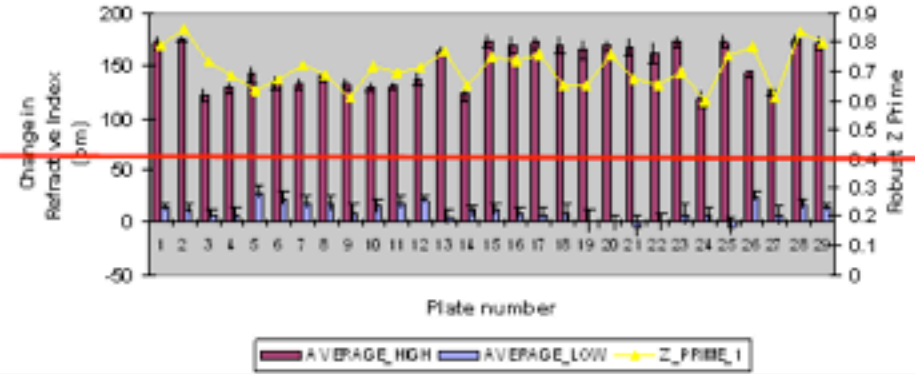
# $\beta$ 2AR assay Validation

VALIDATION n=2, 5uM 1% DMSO

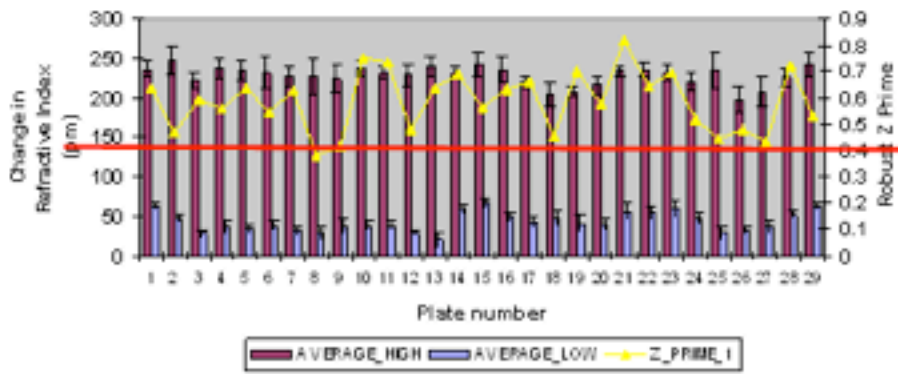
b2AR Agonist Set 1



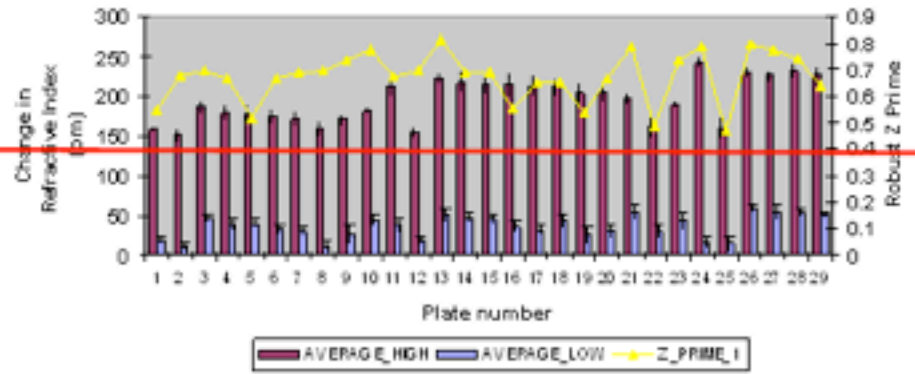
b2AR Agonist Set 2



b2AR Antagonist Set 1

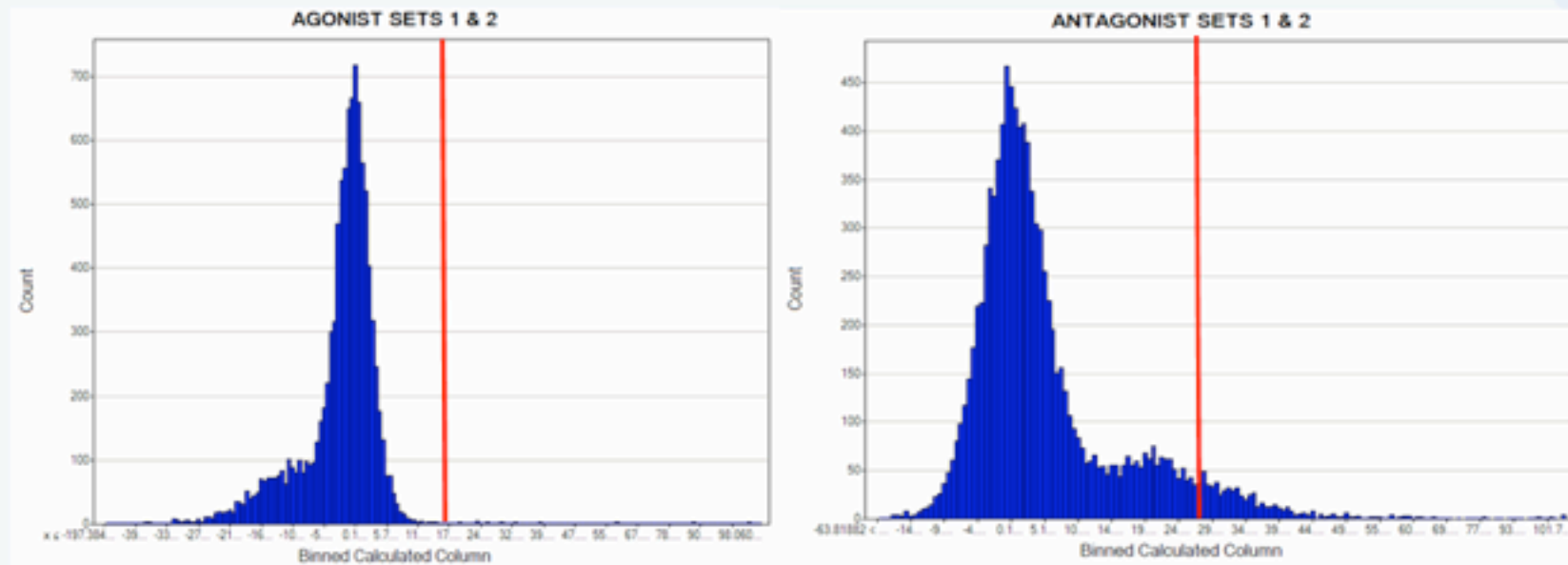


b2AR Antagonist Set 2



# β2AR assay Validation

**VALIDATION n=2, 5uM 1% DMSO – 15 plate batch size**

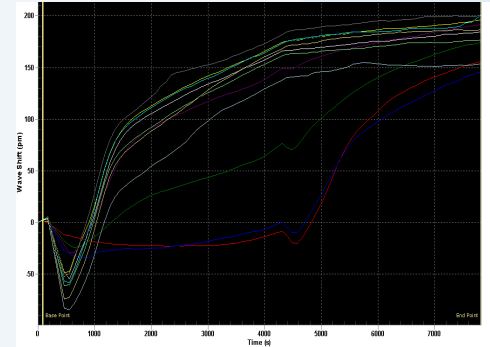


Replicate	# Samples	Rob Cut-off	N hits	HR(%)
b2AR assay agonist Set 1	10,000	16	143	1.50
b2AR agonist assay Set 2		16	133	1.40
b2AR antagonist assay Set 1		31	345	3.60
b2AR antagonist assay Set 2		33	443	4.70

# β2AR assay Pharmacology

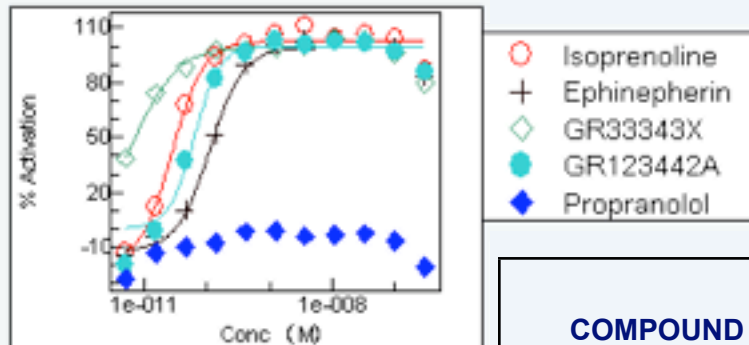
## PHARMACOLOGY – Kinetic read, 1% DMSO

- 3 agonists: Epinephrine, Isoprenaline, GR123442A (fenoterol)
- 1 partial agonist: GR33343X (salmeterol)
- 1 antagonist: GR230382A (propranolol)
- 300nM 1:3 dilutions

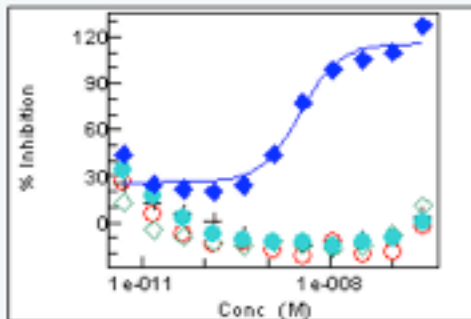


Epinephrine concentration response

Agonist



Antagonist



COMPOUND	PXC50 Label Free Epic A431	PXC50 cAMP Lance CHO	pXC50 cAMP DiscoverX CHO
Isoprenaline - pEC50	10.62	8.30	7.40
Ephinepherin -pEC50	10.01	-	-
GR33343X – pEC50	10.78	9.60	9.60
GR123442A – pEC50	10.18	-	7.20
Propranolol – pIC50	8.43	9.40	9.20

# HIT ID Summary & Key Learnings

- **Summary**
  - High Information (Kinetic) screening allows characterisation of binding/DMR over time with decreased throughput
  - High Throughput (End-point) operation of the Epic System has yielded reproducible hit identification with good quality control in cell based assay, utilising single and dual mode reads

Data generated in collaboration with GlaxoSmithKline