



FLIPR[®] Calcium Assay Kit Update

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April 25, 2009

Agenda



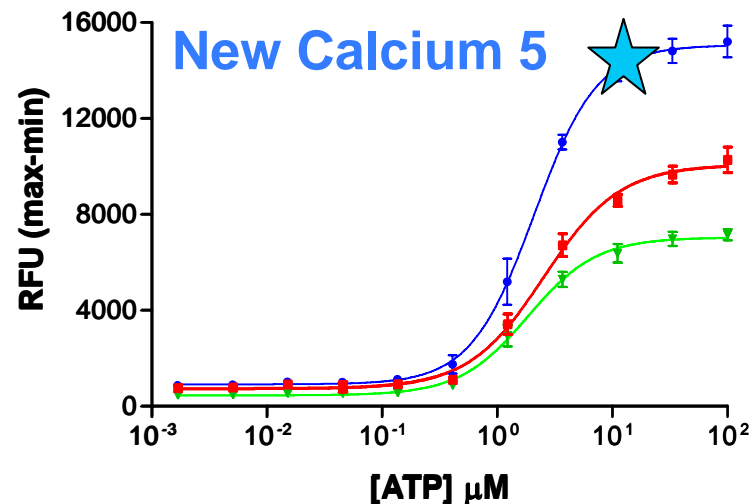
- Current calcium mobilization assay domain
 - Present and future landscape
- Protocols
 - Standard no wash kit protocol
 - Cells
 - CHO M1
 - HEK 293
 - HeLa
 - Jurkat
- Lots of data
 - Transfected receptors
 - Endogenous receptors
 - Difficult receptors
- Summary

Current calcium mobilization assay domain



- 2006 FLIPR[®] Calcium 4 Kit
- New competitive reagents
- Looking to the Future
 - New FLIPR[®] Calcium 5 Kit

**ATP Agonism of P2Y Receptor
in HEK 293 Cells**



New FLIPR[®] Calcium 5 Kit



- What remains the same?
 - Assay protocol
 - Homogenous assay
 - Consistent pharmacology
 - No wash steps, or media removal required
 - Same great masking dye performance
- What is new?
 - Superior performance Calcium indicator technology
 - Higher signal response
 - Larger signal to noise ratio
 - Improved Z factors

Assay Protocol



- Cells plated overnight @ 37°C, 5% CO₂ in B/C TC coated 384-well plates
 - CHO M1 @ 10,000/well
 - HEK-293 @ 15,000/well in poly d-lysine coated plates
 - HeLa @ 10,000/well
 - Jurkat @ 75,000/well
- Dyes made up in HBSS + 20 mM HEPES, pH 7.4
 - Probenecid
 - CHO M1
 - HeLa
 - Jurkat
 - No Probenecid
 - HEK-293
- 25 μL dye added to 25 μL cells in wells
- Plates incubated 45 minutes @ 37°C
- Plates incubated at 15 minutes room temp prior to assay

FLIPR^{TETRA} Protocol



- Excitation and Detection
 - Excitation Calcium LEDs 470-495 nm
 - Emission Filters 514-575 nm
- ICCD camera settings
 - 6% Gate
 - Gain 2000 (fixed)
 - 0.53 second exposure
 - 1 second interval
- Fluid transfer
 - Volume - 12.5 μL
 - Height – 40 μL
 - Speed – 30 $\mu\text{L}/\text{sec}$

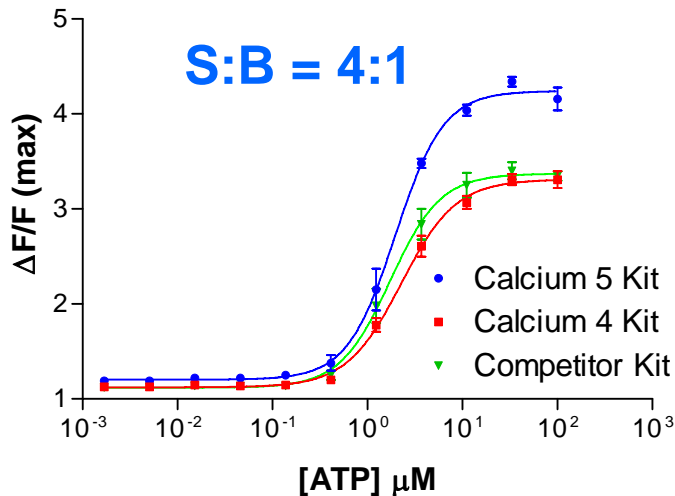


HEK 293 Cells



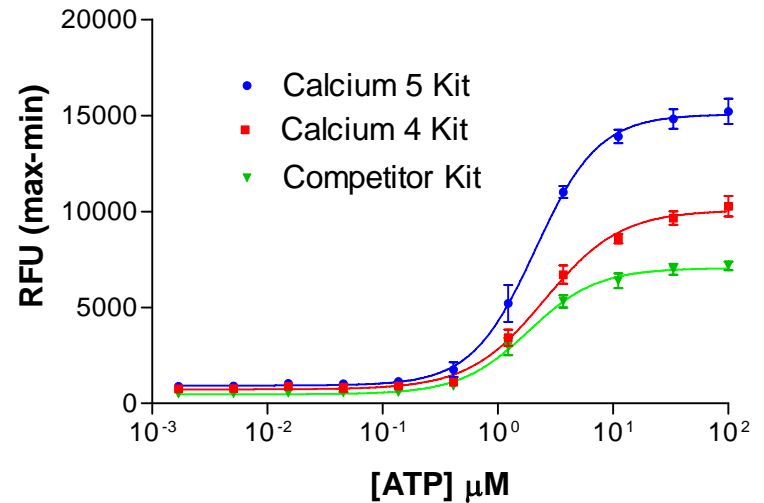
- P2Y endogenous receptor
- Common cell line for transfected receptors
- Delicate monolayer benefits from no-wash protocol

Agonism of P2Y Receptor by ATP in HEK 293 Cells



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ATP Agonism of P2Y Receptor in HEK 293 Cells

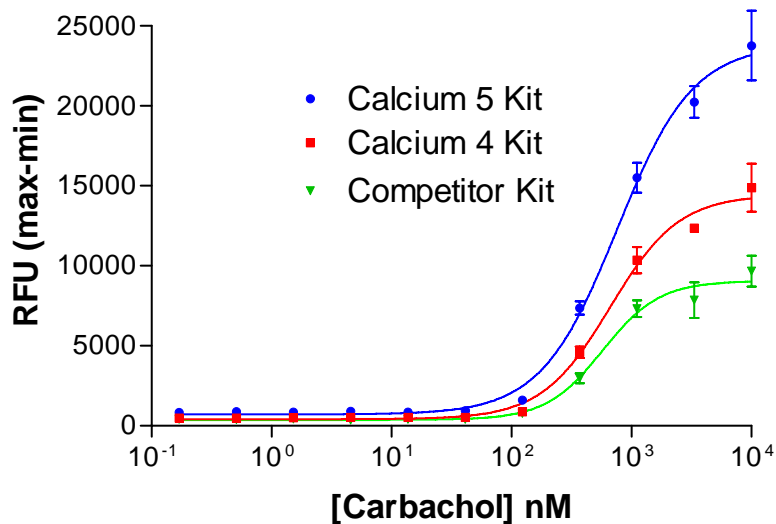


ATP uM	Cal 4 Kit	Cal 5 Kit	Competitor
EC ₅₀	2.55	2.11	1.85
Z at EC ₈₀	0.67	0.88	0.78

HEK 293 - Endogenous M3 Receptor

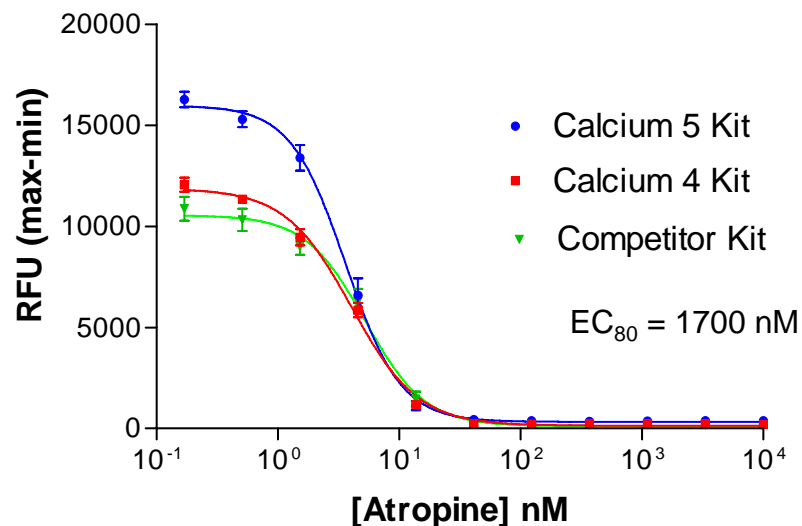


Carbachol Agonism of Endogenous M3 Receptor in HEK 293 Cells



Carbachol nM	Cal 4 Kit	Cal 5 Kit	Competitor
EC ₅₀	667.1	768.8	576.3
Z at EC ₈₀	0.73	0.79	0.76

Atropine Antagonism of Muscarinic M3 Response to Carbachol in HEK 293 Cells

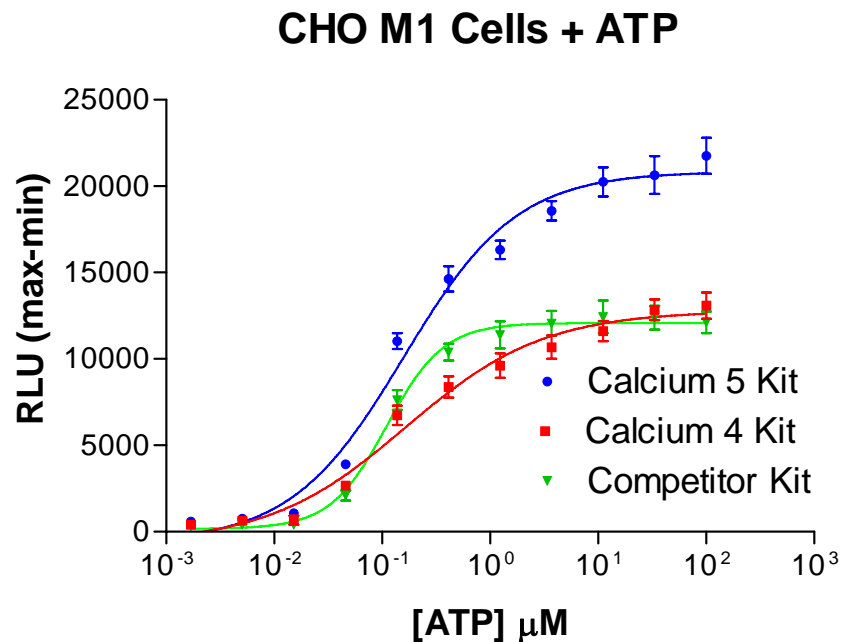
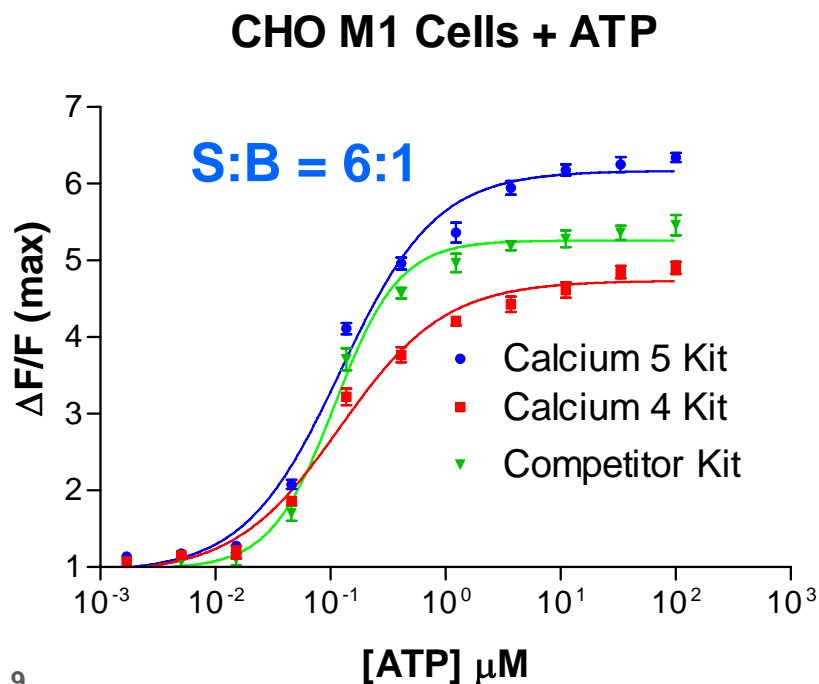


Atropine nM	Cal 4 Kit	Cal 5 Kit	Competitor
IC ₅₀	4.05	3.60	5.40
Z at IC ₅₀	0.83	0.84	0.84

CHO-M1 Cells



- P2Y Endogenous Receptor
- Common cell line
- Transfected receptor

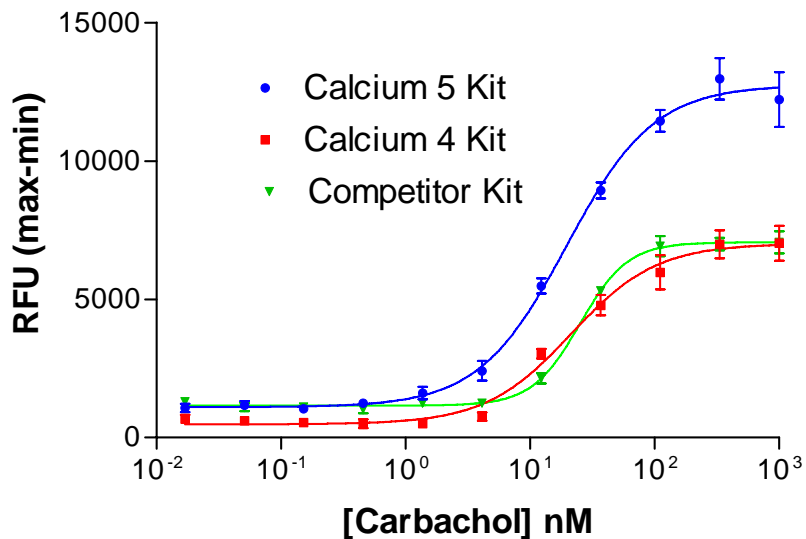


ATP μM	Ca4	Ca5	Competitor
EC ₅₀	0.17	0.16	0.14
Z at EC ₈₀	0.74	0.83	0.83

CHO M1 Cells

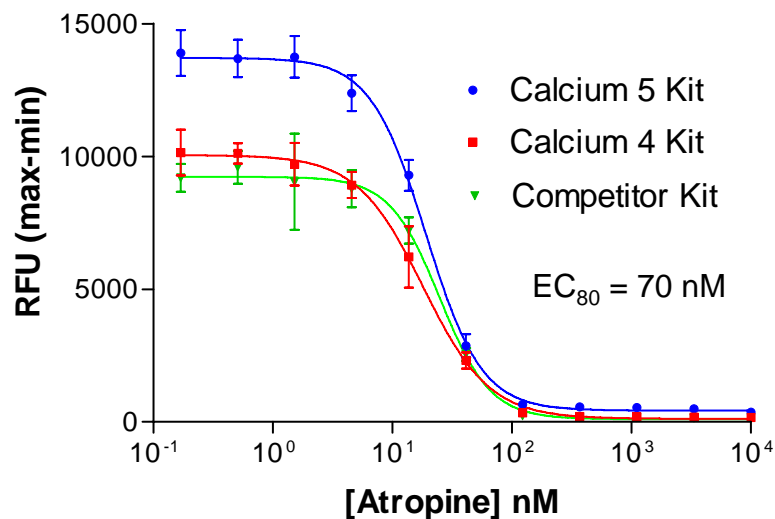


Carbachol Agonism in CHO-M1 Cells



Carbachol nM	Cal 4 Kit	Cal 5 Kit	Competitor
EC ₅₀	21.4	19.65	25.19
Z at EC ₈₀	0.59	0.74	0.8

Atropine Antagonism of Carbachol in CHO M1 Cells



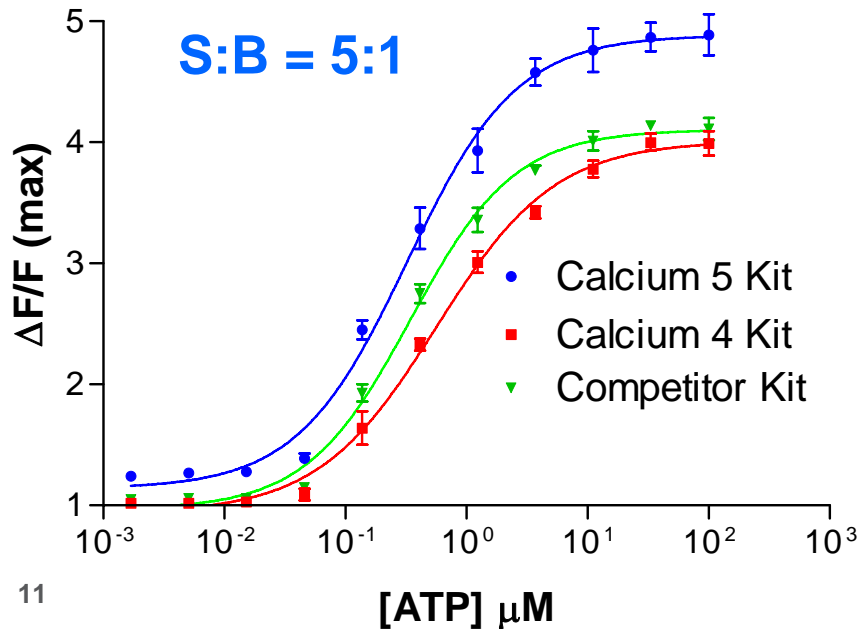
Atropine nM	Cal 4 Kit	Cal 5 Kit	Competitor
IC ₅₀	18.2	19.13	24.99
Z at IC ₅₀	0.8	0.77	0.77

HeLa Cells

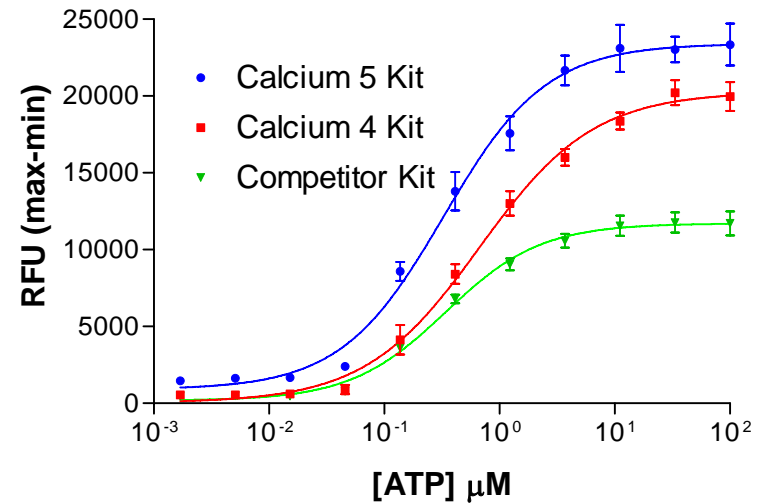


- Endogenous P2Y receptor
- Calcium 5 Kit 2X Signal Window over competitor kit

HeLa Cells + ATP



ATP Agonism of P2Y Receptor in HeLa Cells



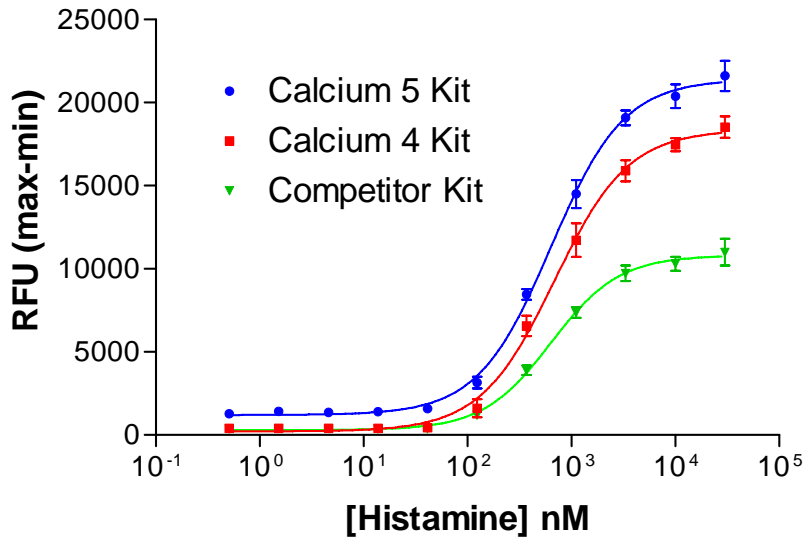
ATP nM	Cal 4 Kit	Cal 5 Kit	Competitor
EC ₅₀	0.67	0.33	0.34
Z at EC ₈₀	0.74	0.68	0.84

HeLa Cells



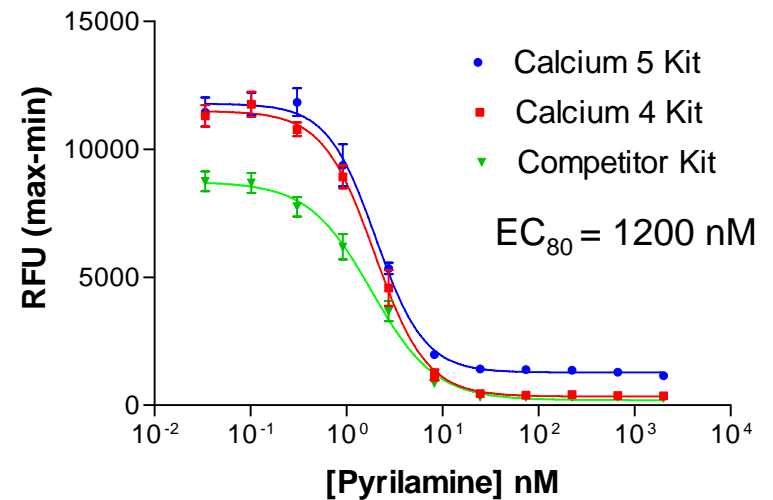
- Endogenous Histamine H1 receptor

Endogenous Histamine H1 Response in HeLa Cells



Histamine nM	Cal 4 Kit	Cal 5 Kit	Competitor
EC ₅₀	688.3	635.6	637.5
Z at EC ₈₀	0.72	0.79	0.85

Pyrilamine Antagonism of Endogenous Histamine H1 Response in HeLa Cells



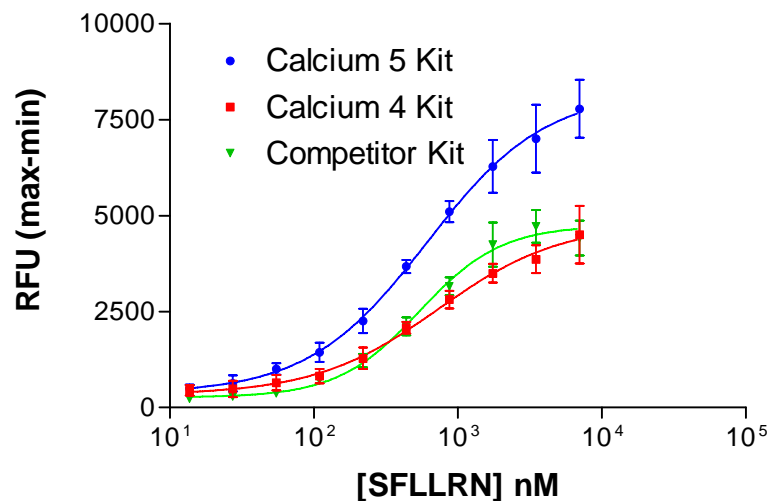
Pyrilamine nM	Cal 4 Kit	Cal 5 Kit	Competitor
IC ₅₀	1.965	2.02	1.83
Z at IC ₅₀	0.82	0.67	0.73

JURKAT Data

- Non-adherent cell line
- Endogenous receptor
- Sticky peptides
- Worst case scenario



SFLLRN Agonism of Thrombin Receptor in Jurkat T Cell Line

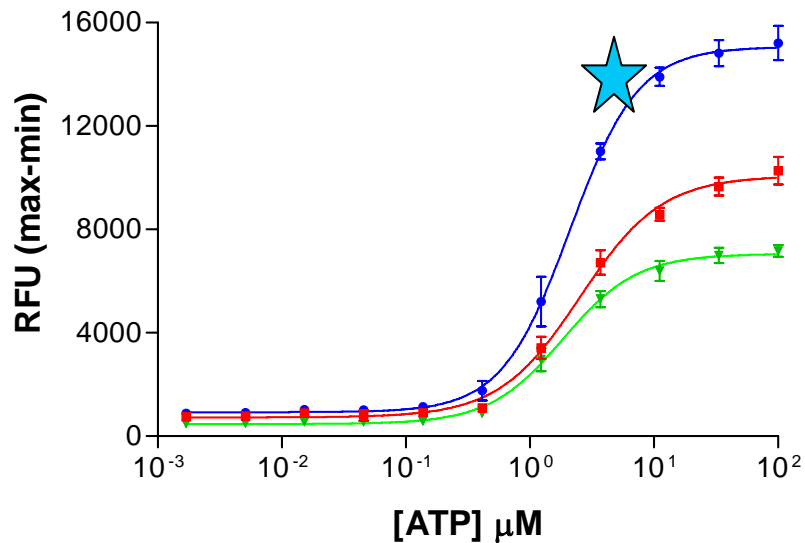


SFLLRN nM	Cal 4 Kit	Cal 5 Kit	Competitor
EC ₅₀	732	609	541
Z at EC ₅₀	0.62	0.76	0.72

FLIPR[®] Calcium 5 Kit



ATP Agonism of P2Y Receptor in HEK 293 Cells



- Novel indicator provides larger signal window
 - Allosteric modulators
 - Low receptor expression
 - Difficult targets
- Proven quench technology
 - Lowers background noise
- No wash system
 - Non-adherent cells
 - Cells with adherence issues

Summary



Let data speak for itself
If you'd like to evaluate our
New FLIPR Calcium 5 Assay Kit

Available this July

For more information
Please visit us at SBS
Booth # 76

Acknowledgments



- European Applications Team
 - Simon Lydford
 - Cerys Huggins, PhD.
- Sunnyvale Applications Team
 - Ming Yu, PhD.
 - Bingfang Huan, PhD.
 - Trisha Tutana
 - Kalpana Pachipala