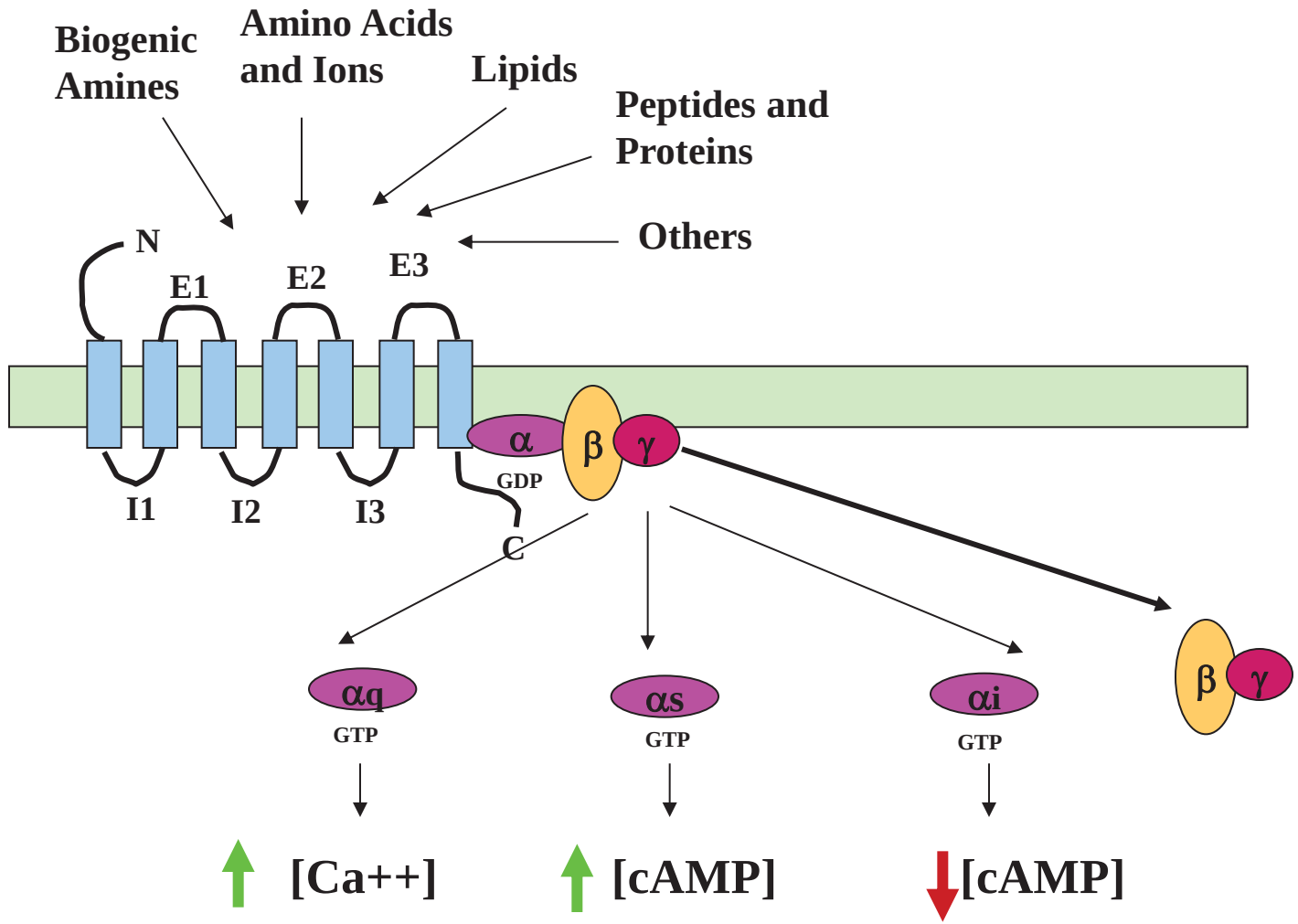
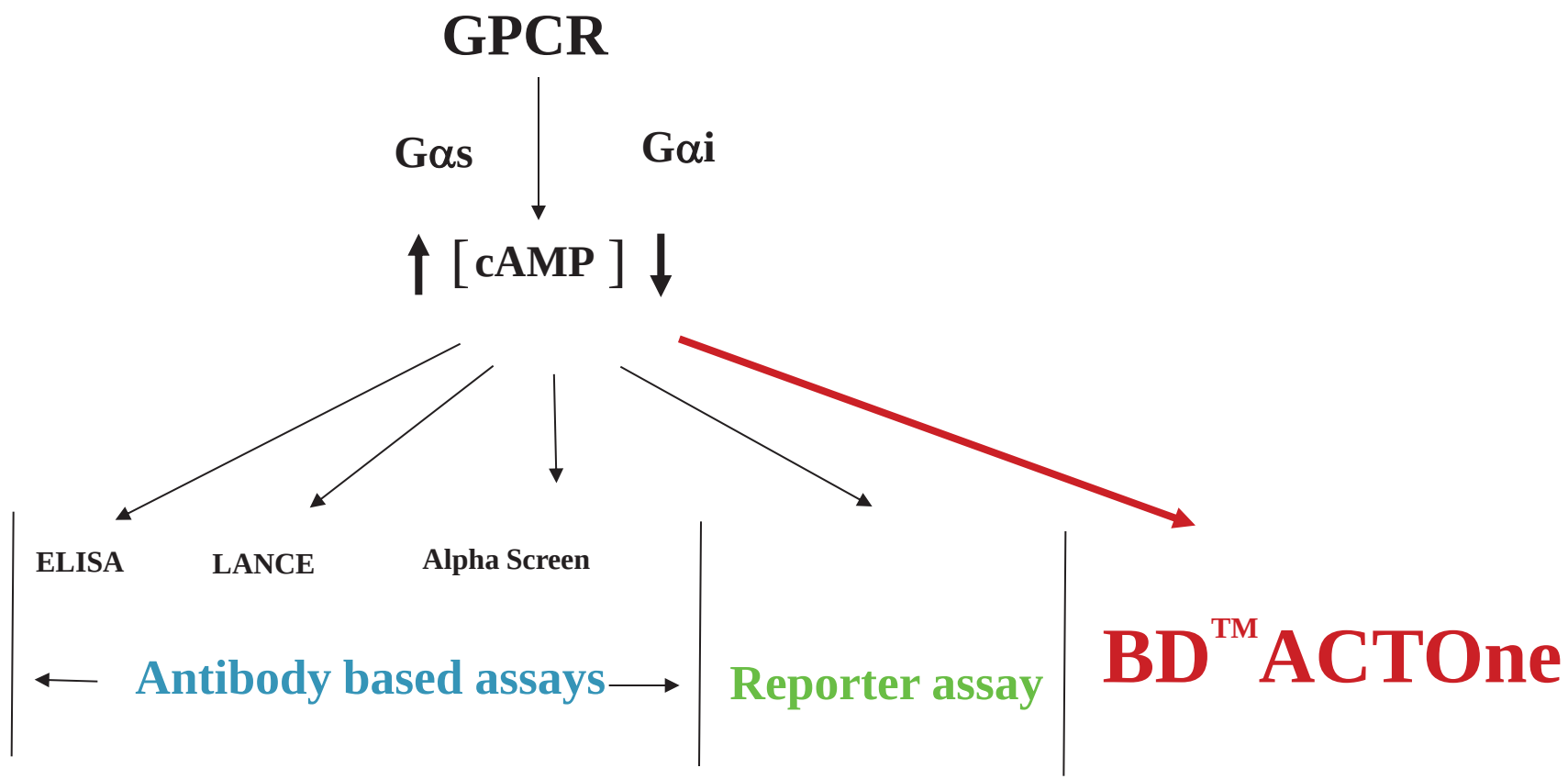
Advanced cAMP Technology (ACTOne) Assay on G-Protein Coupled Receptors (GPCRs)

Signal Transduction Pathway with GPCR



cAMP Assays Available on Market



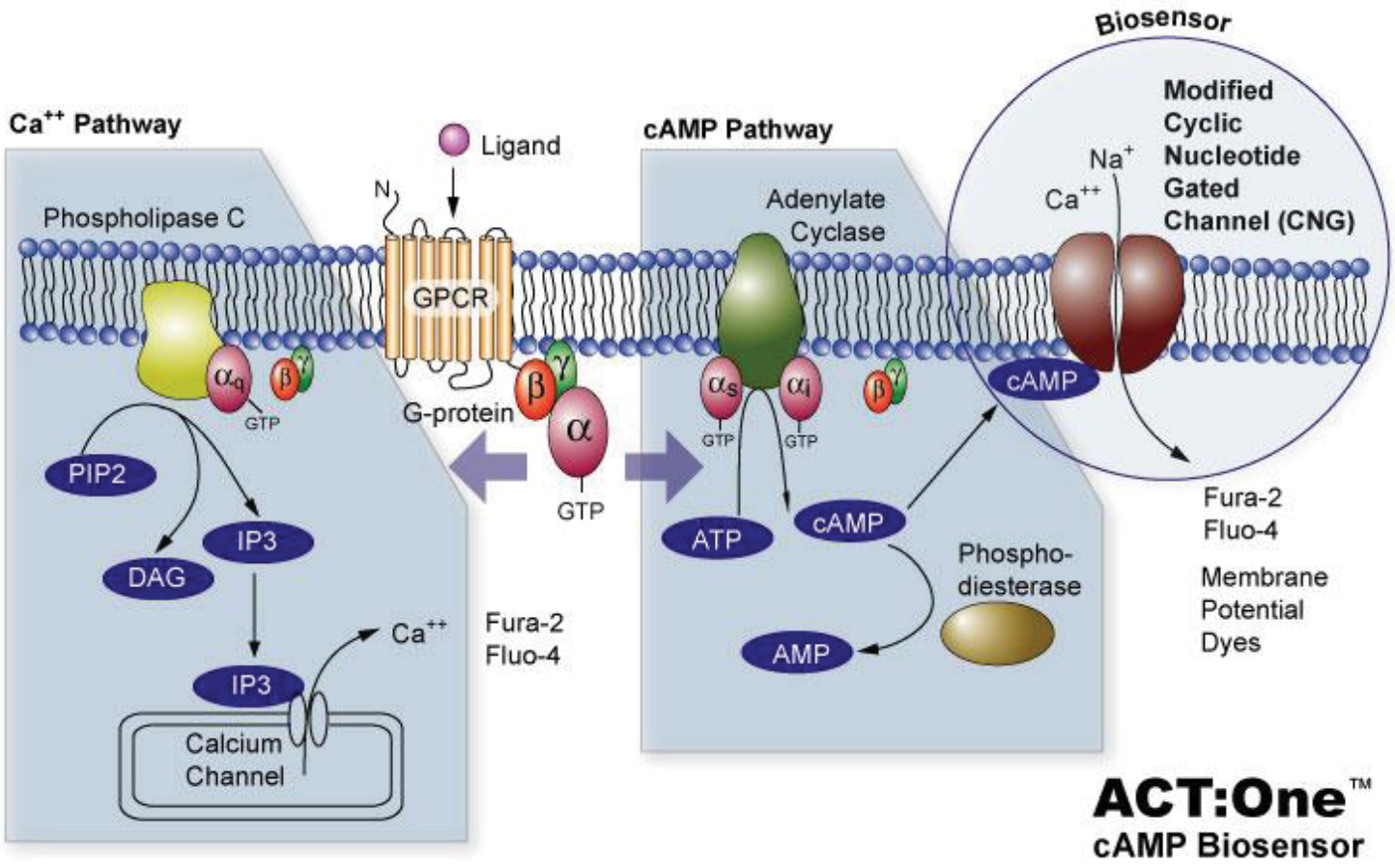
LANCE: A homogenous TR-FRET assay

Alpha Screen: A bead based non-radioactive **A**mplified **L**uminescent **P**roximity **H**omogeneous **A**ssay

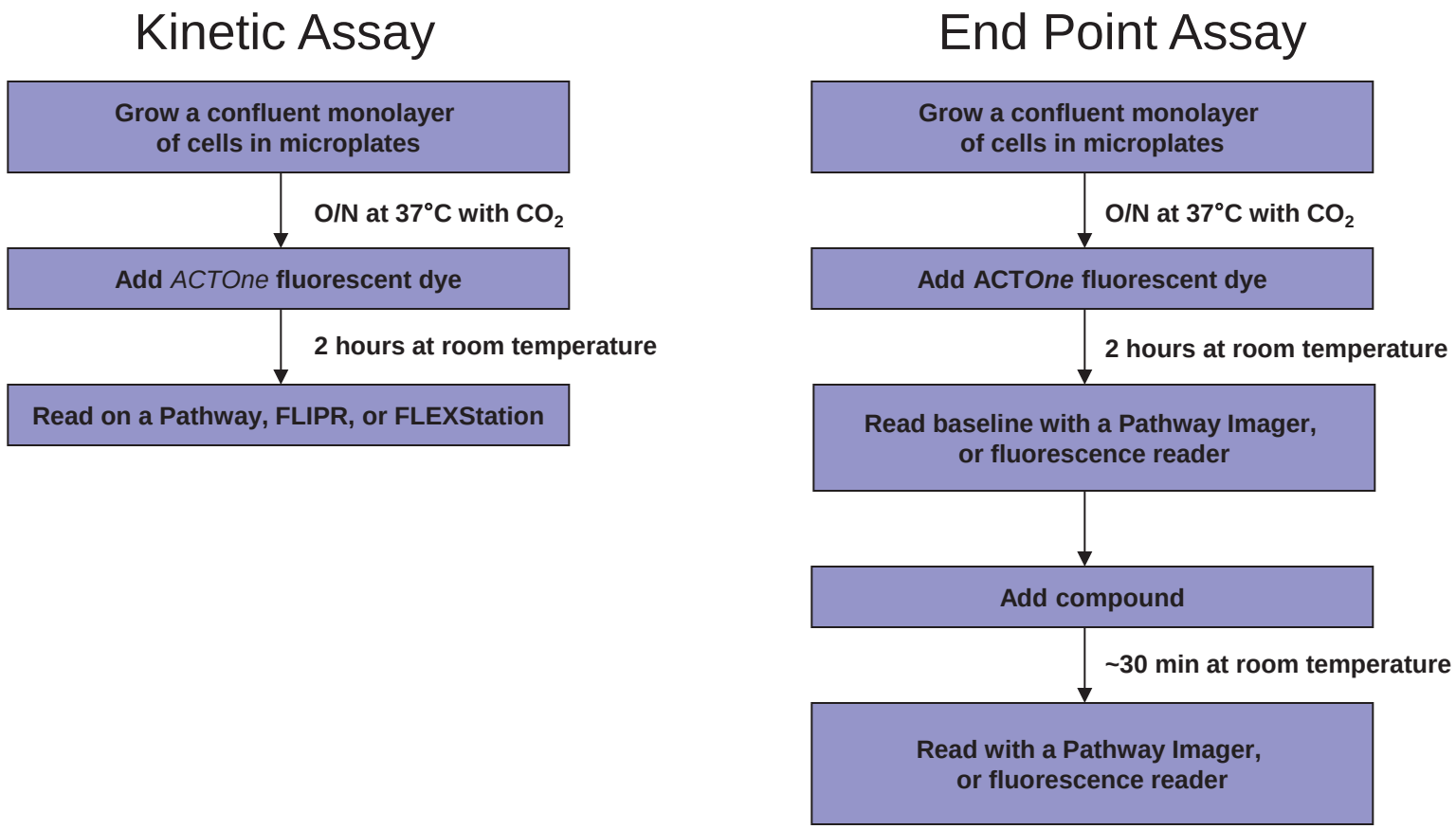
BD™ACTOne

- Living cell
- Real time
- High Sensitivity
- Big assay window
- World wide patent issued
- Well accepted by pharmaceutical industry

Overview of ACT*One* Biosensor



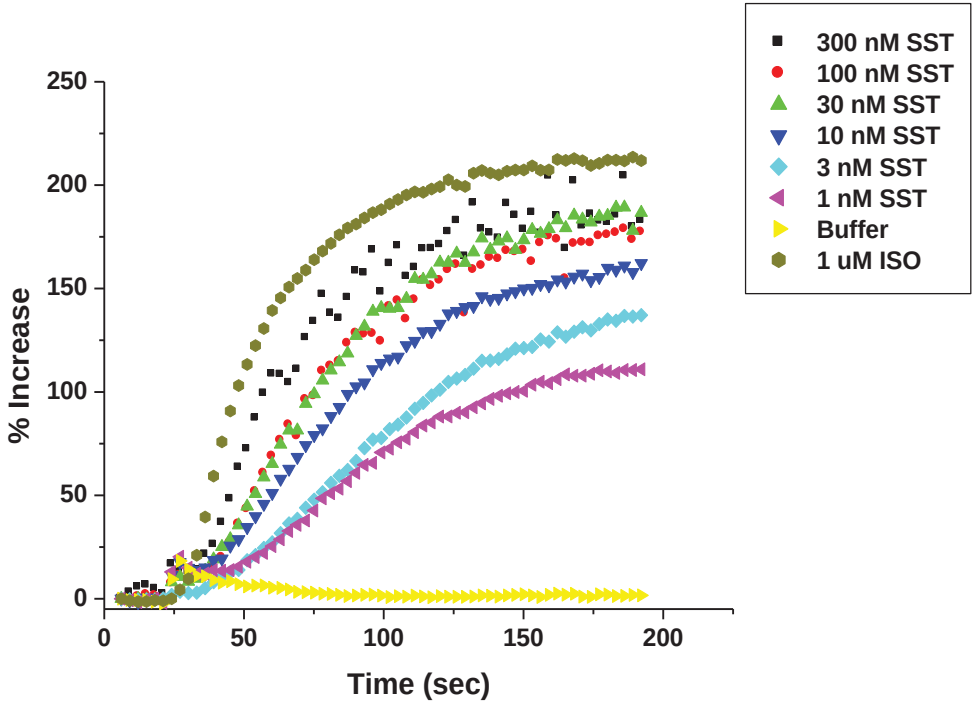
ACTOne Assay Flow Chart



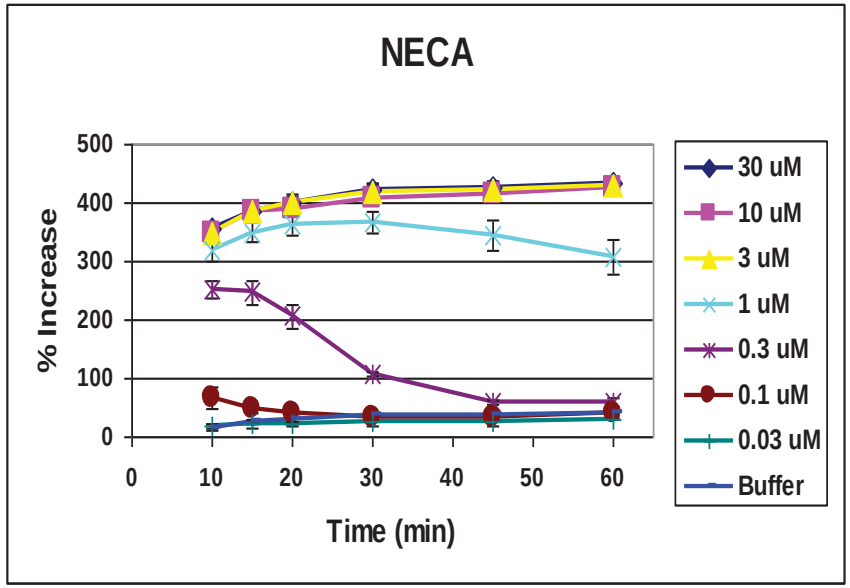
The BD Act*One* assay is a homogenous assay without washing steps. The assay can be performed as an end point assay or a kinetic assay.

ACTOne: Fast Response and Stable Signal

Kinetic Assay

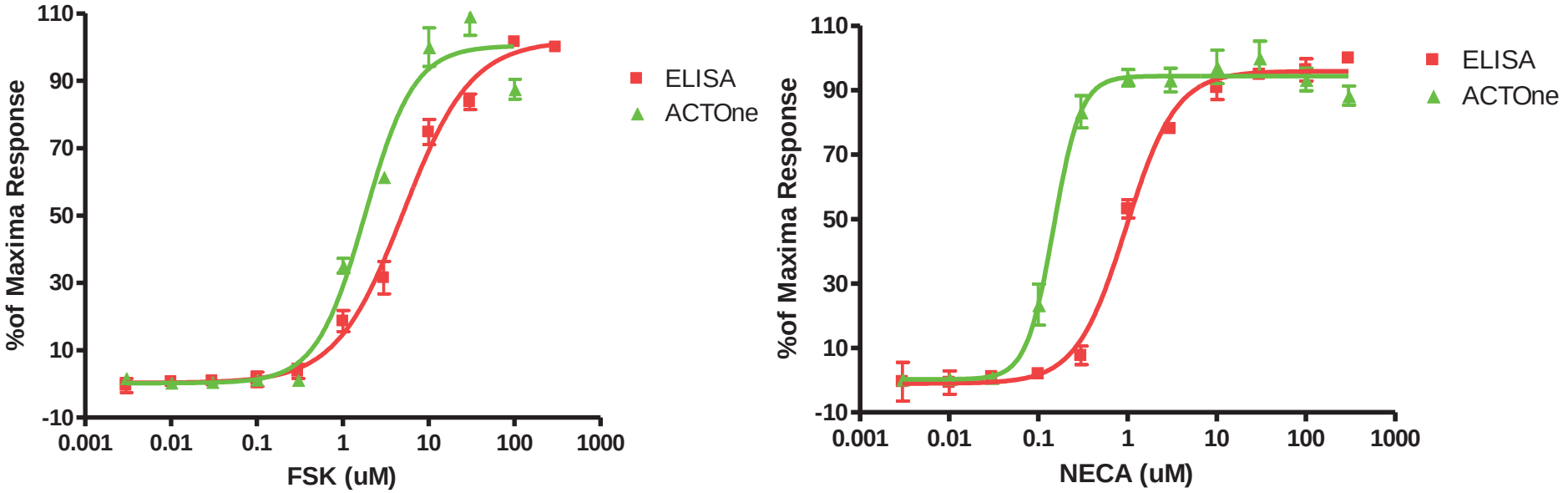


End Point Assay



SST = somatostatin, agonist of SSTR
NECA: Agonist of A2b

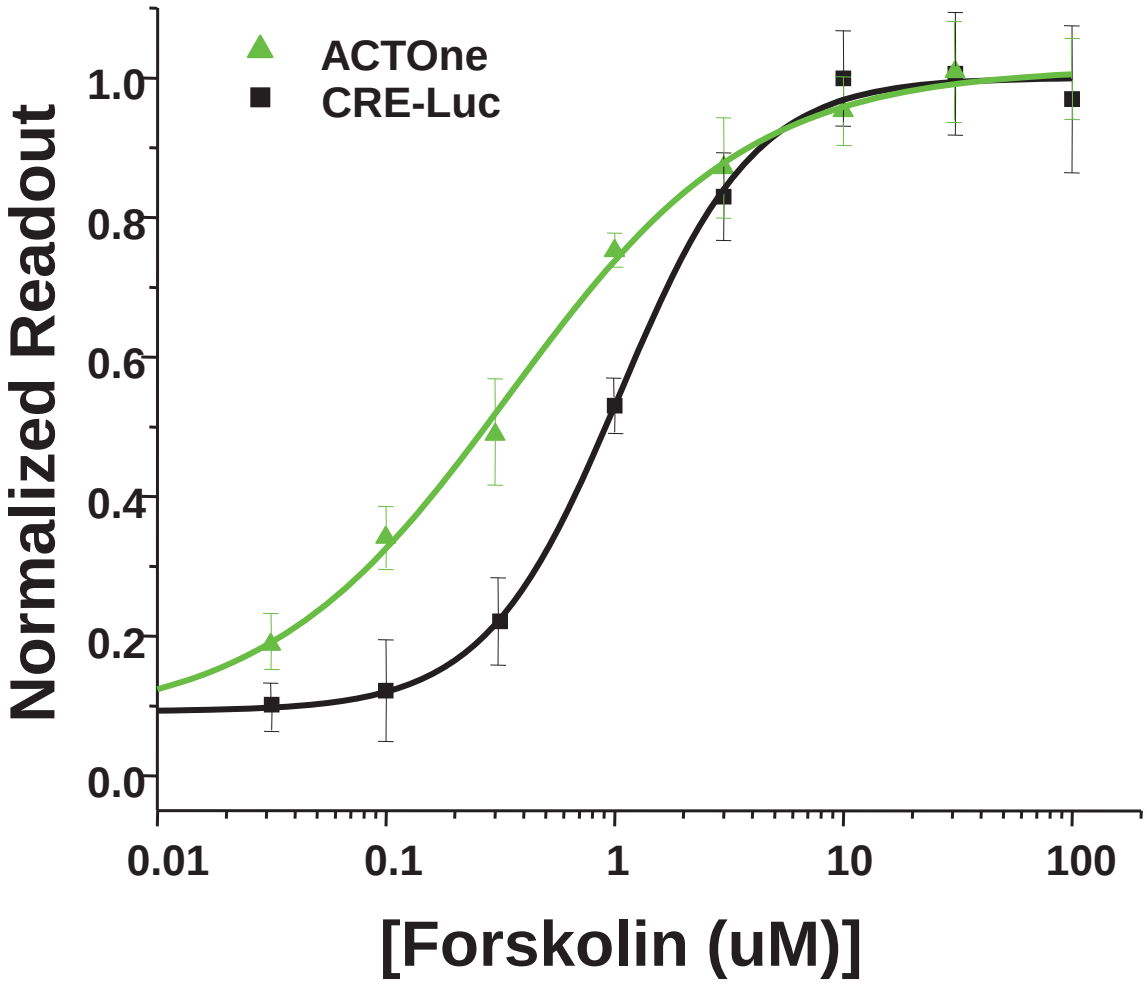
ACT*One* Has Higher Sensitivity Over ELISA



Act*One* technology shows improved sensitivity compared to ELISA by measuring localized cAMP generation at the cytoplasmic membrane.

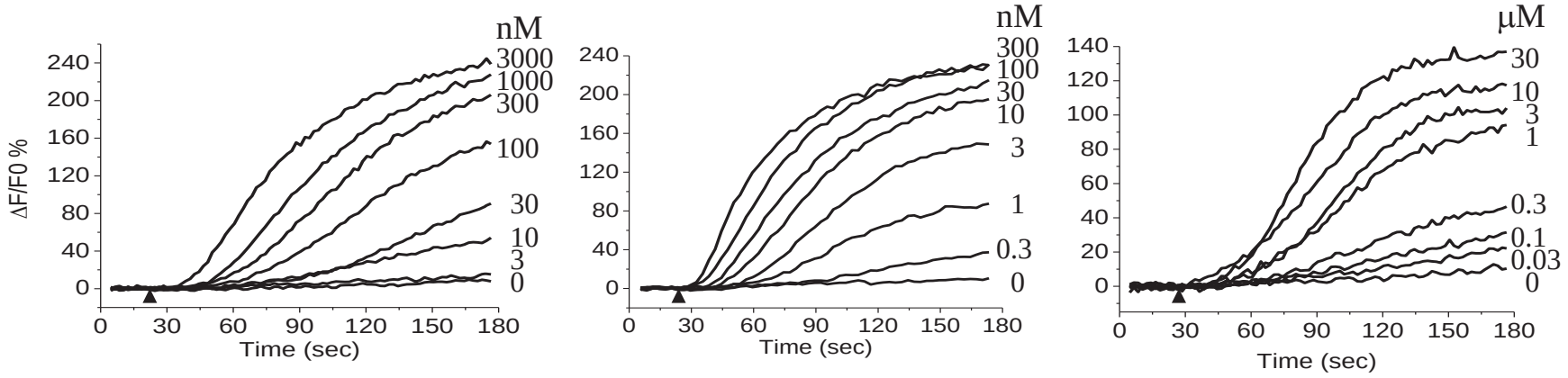
FSK: Foskolin, Activator of adenylate cyclase (***AC***)

ACT*One* Is More Sensitive Than Reporter Assay

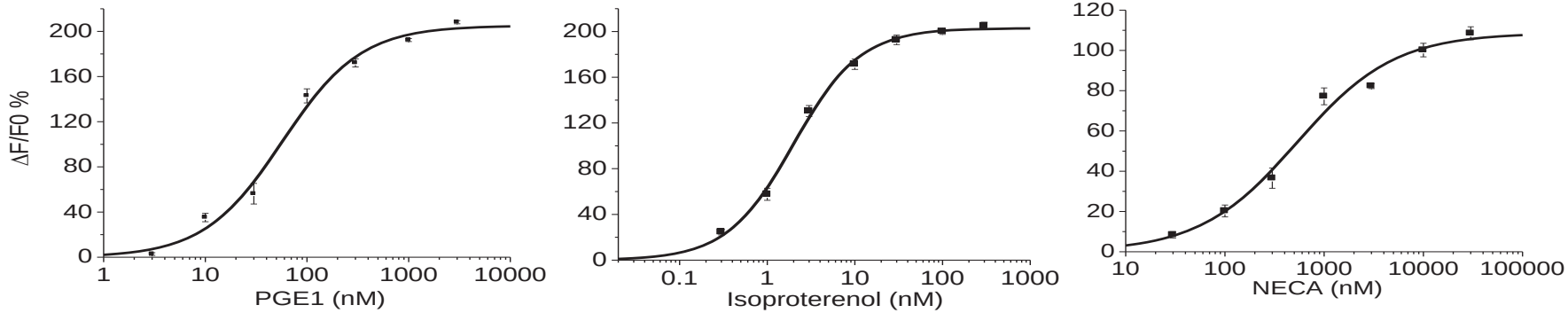


ACTOne is Excellent to Detect Endogenous GPCRs

Ligand: **PGE1** **Isoproterenol** **NECA**

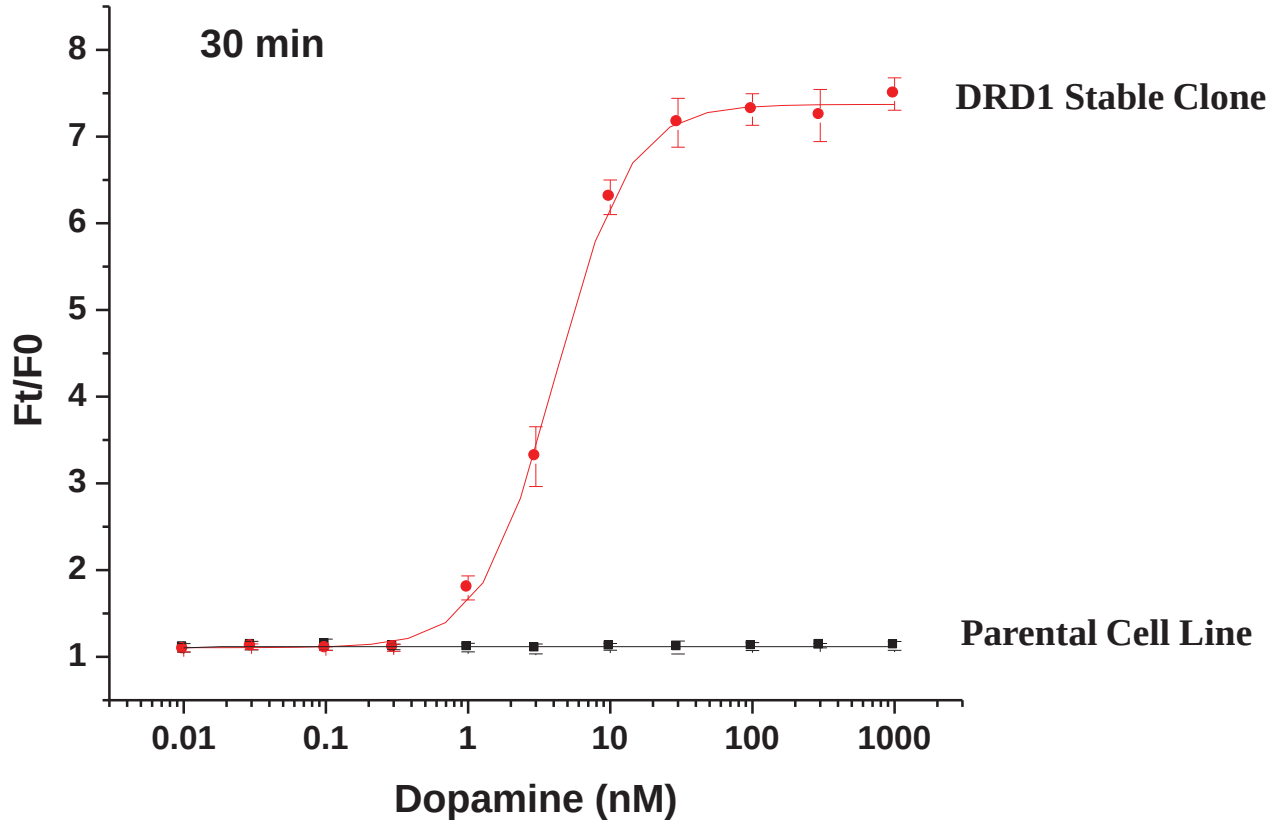


Receptor: **Prostanoid EP2** **β-adrenoceptor** **Adenosine A2B**

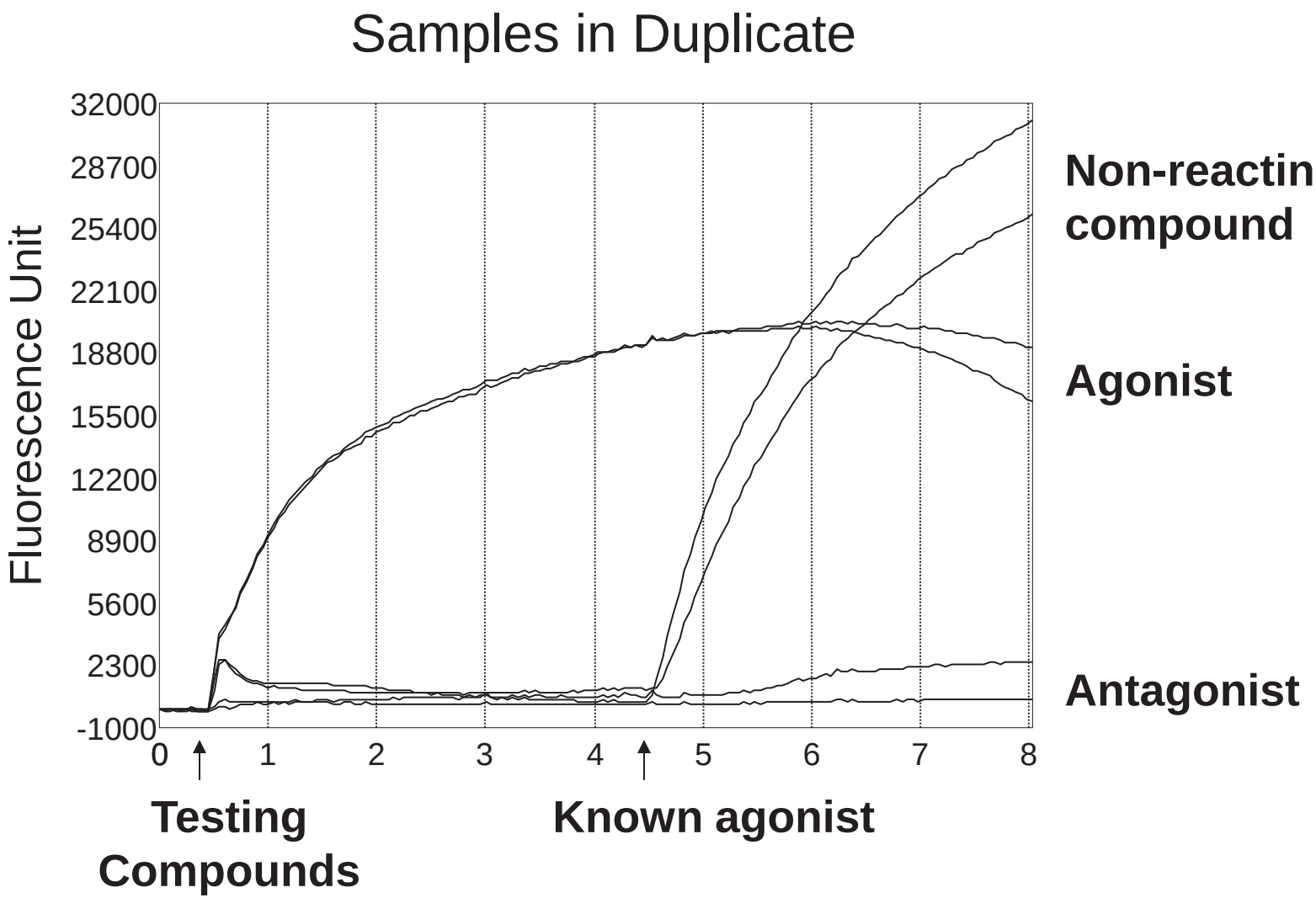


EC50 = 57 nM **EC50 = 2.0 nM** **EC50 = 540 nM**

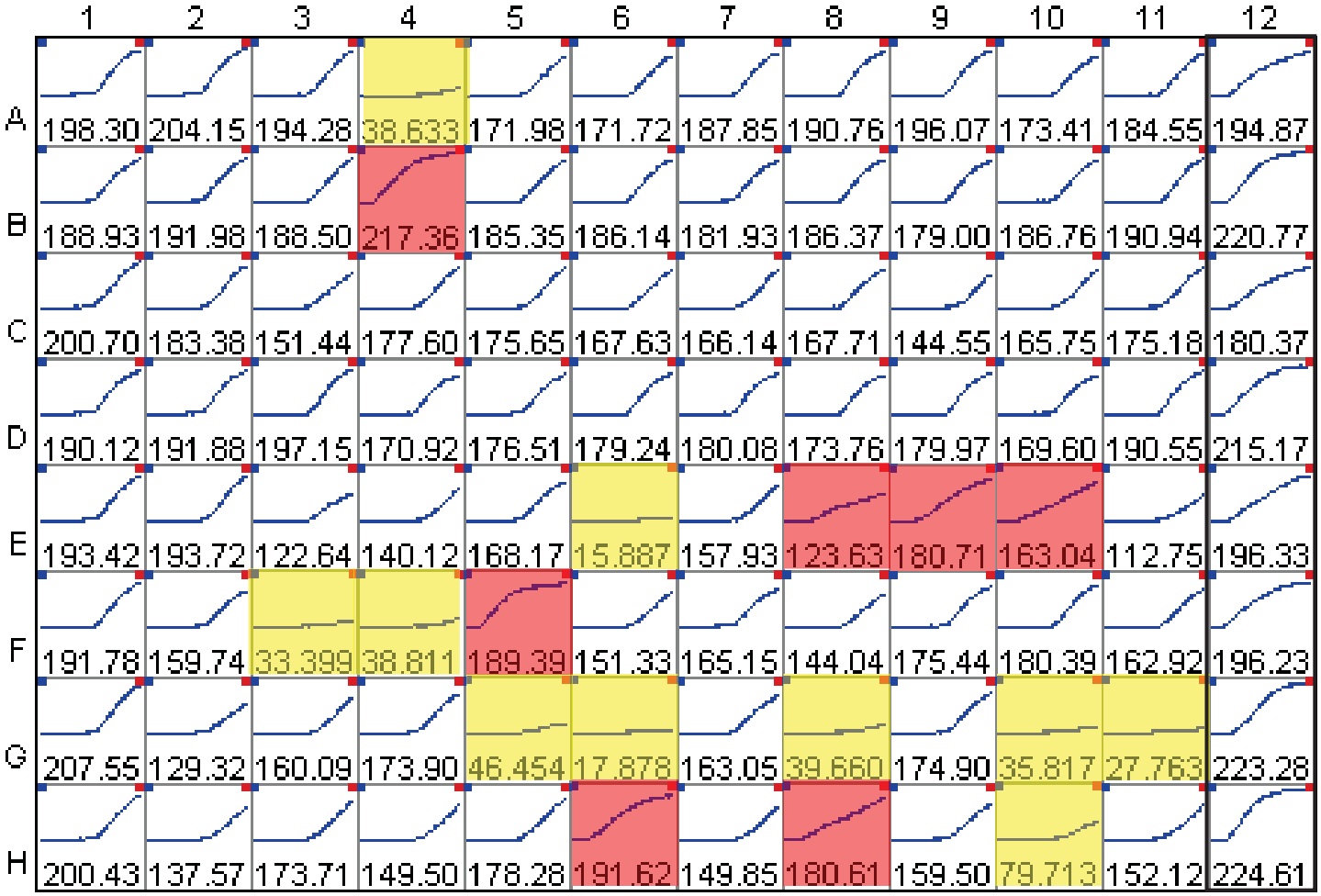
ACTOne Works Well with Recombinant Gs-GPCR



ACTOne Can Screen Both Agonist and Antagonist in One Assay



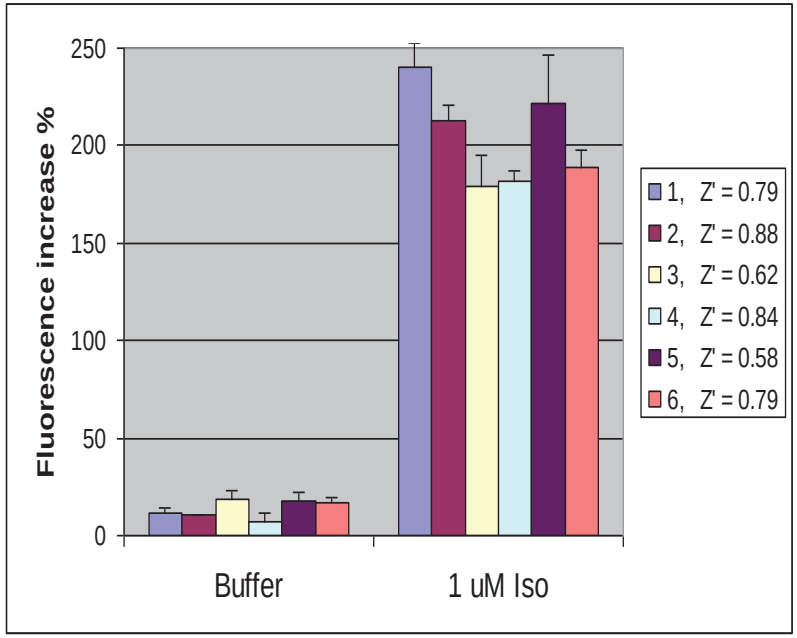
β-Adrenoceptor Agonist and Antagonist Detection



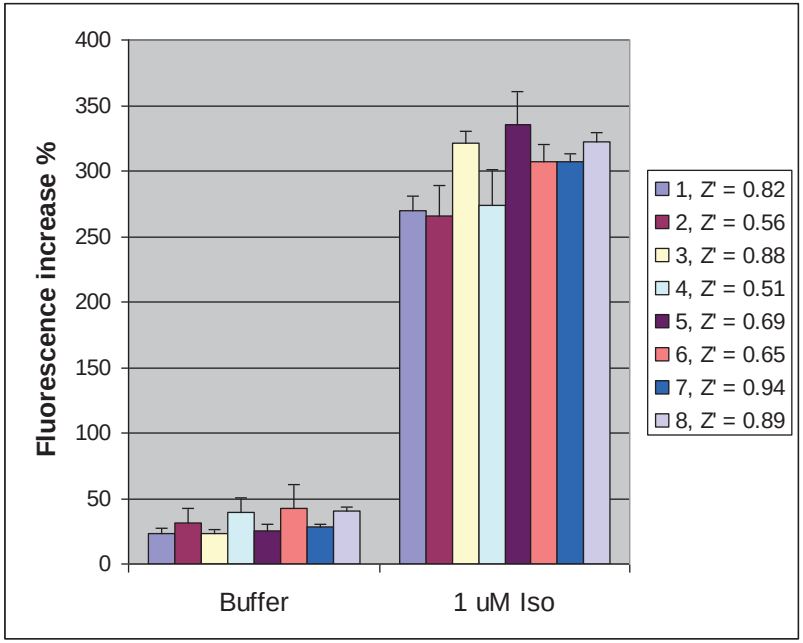
ACTOne: Excellent S/N Ratio and Reproducibility

Well-to-well and Experiment-to-experiment Variability

Kinetic Assay



Endpoint Assay



Z' = 1- 3*(SD1+SD2)/(Fmax-Fmin)

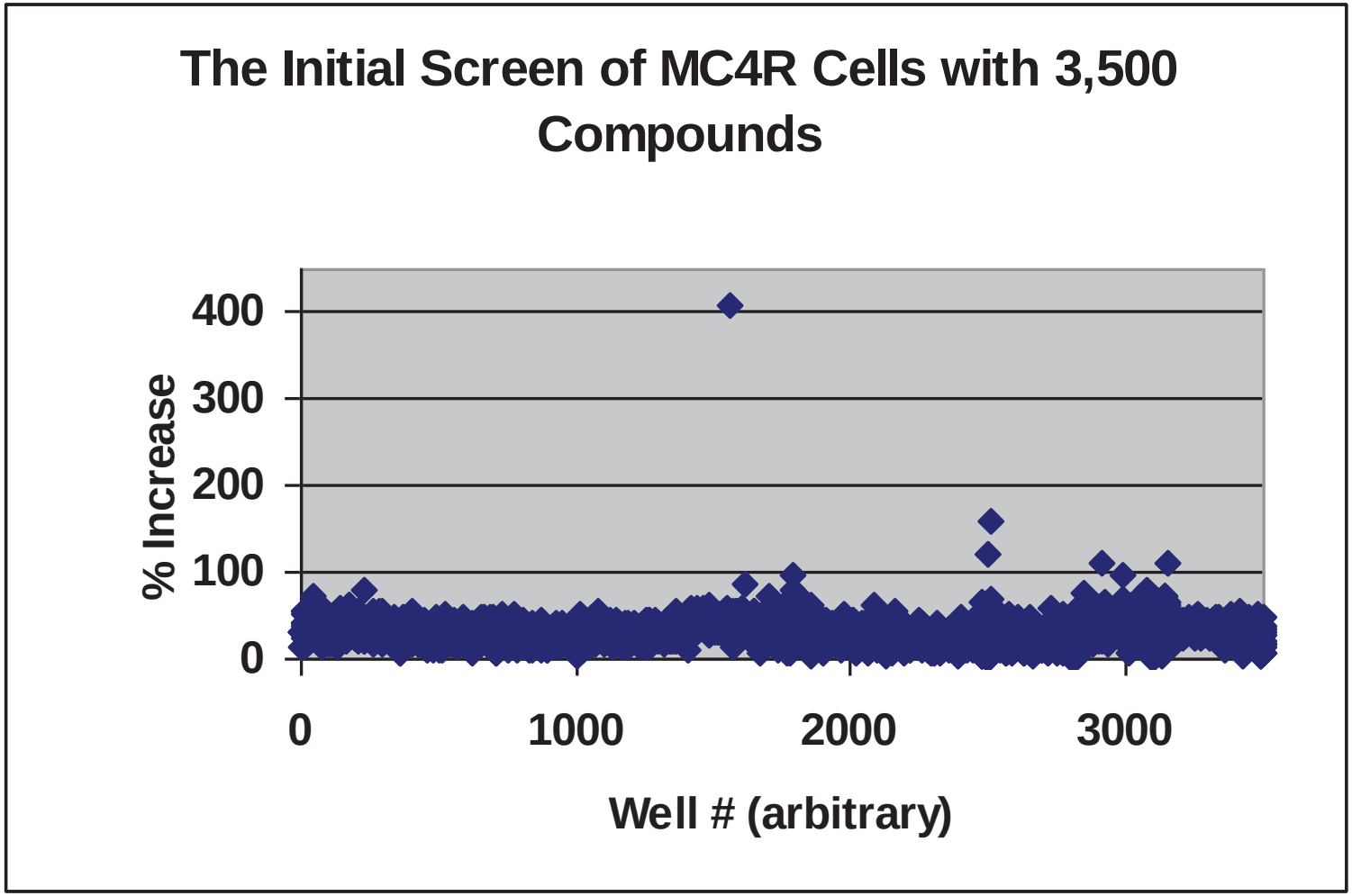
Z' = 1.0: Ideal. This is approached when you have a huge dynamic range with tiny standard deviations.
Z-factors can never actually equal 1.0 and can certainly never be greater than 1.0.

Z' between 0.5-1.0: An excellent assay.

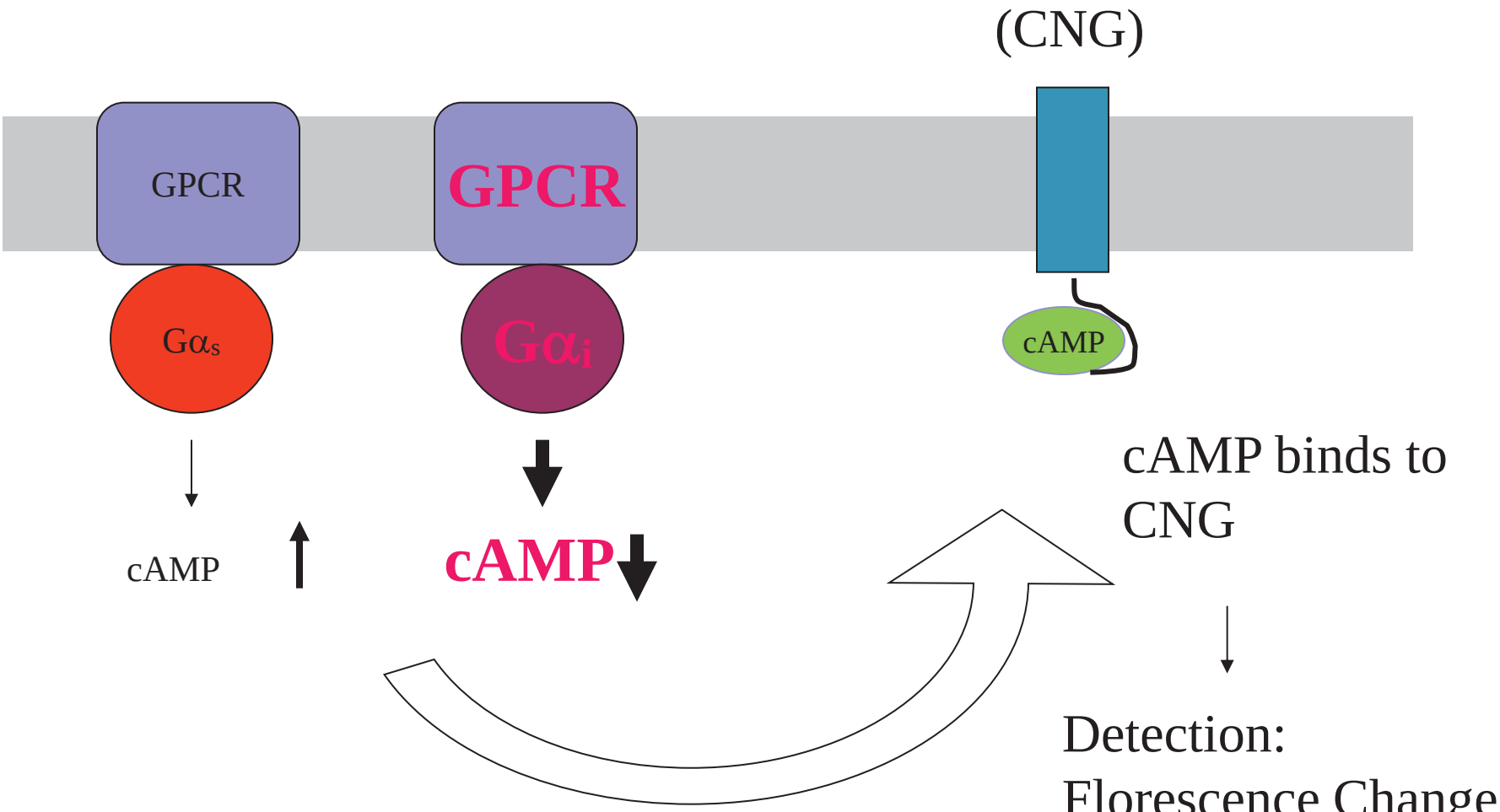
Z' between 0-0.5: A marginal assay.

Z' less than 0: The signal from the positive and negative controls overlap, making the assay essentially useless for screening purposes.

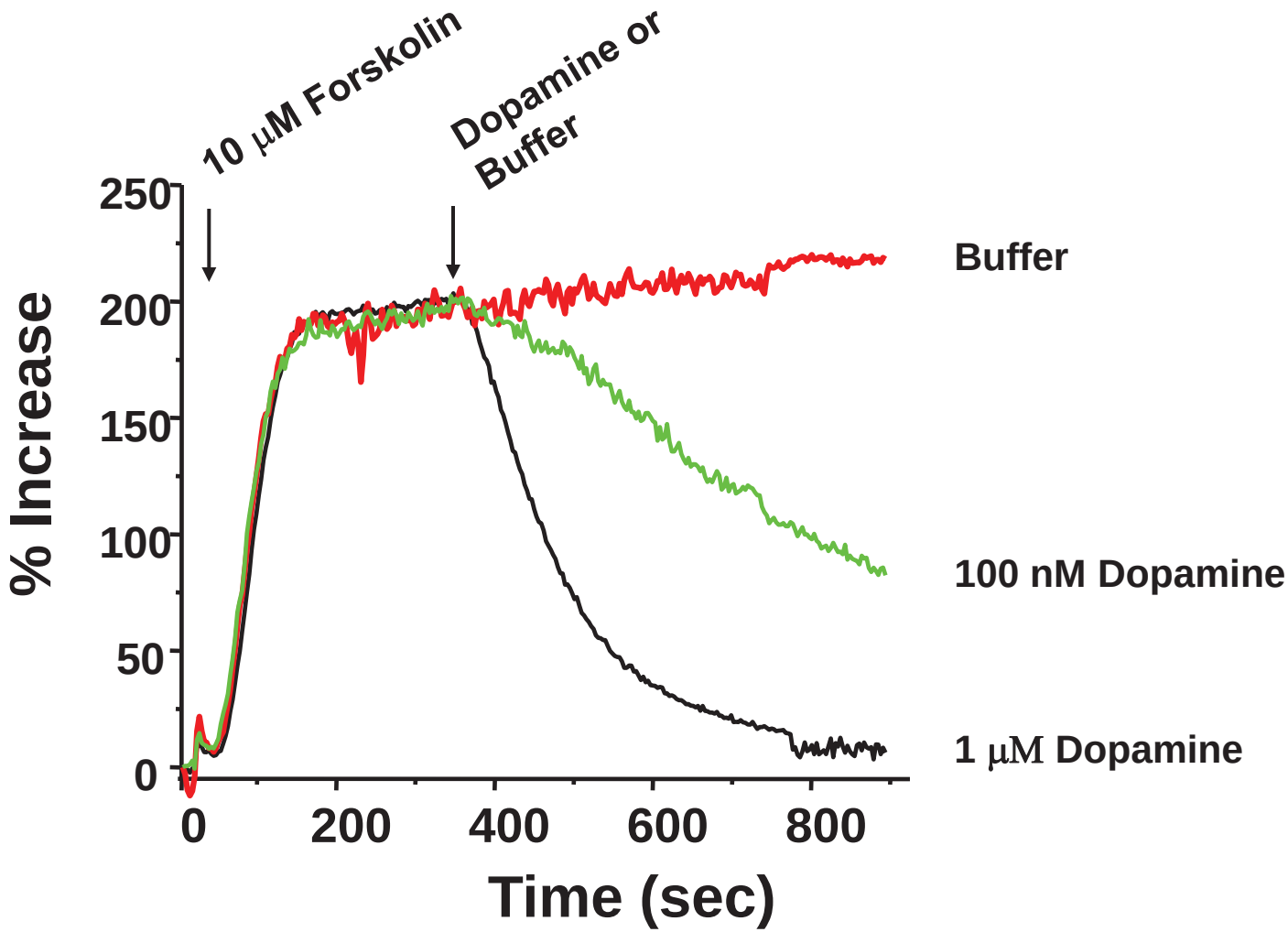
ACTOne on High Throughput Screening



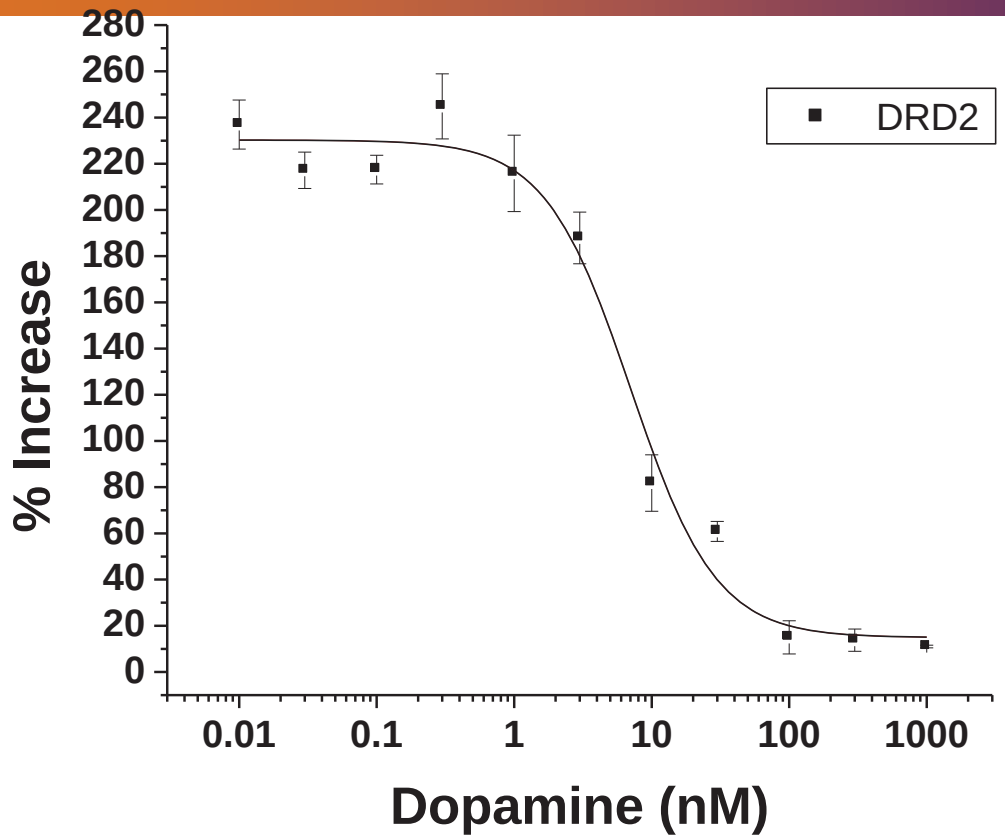
ACTOne for Gi-GPCR



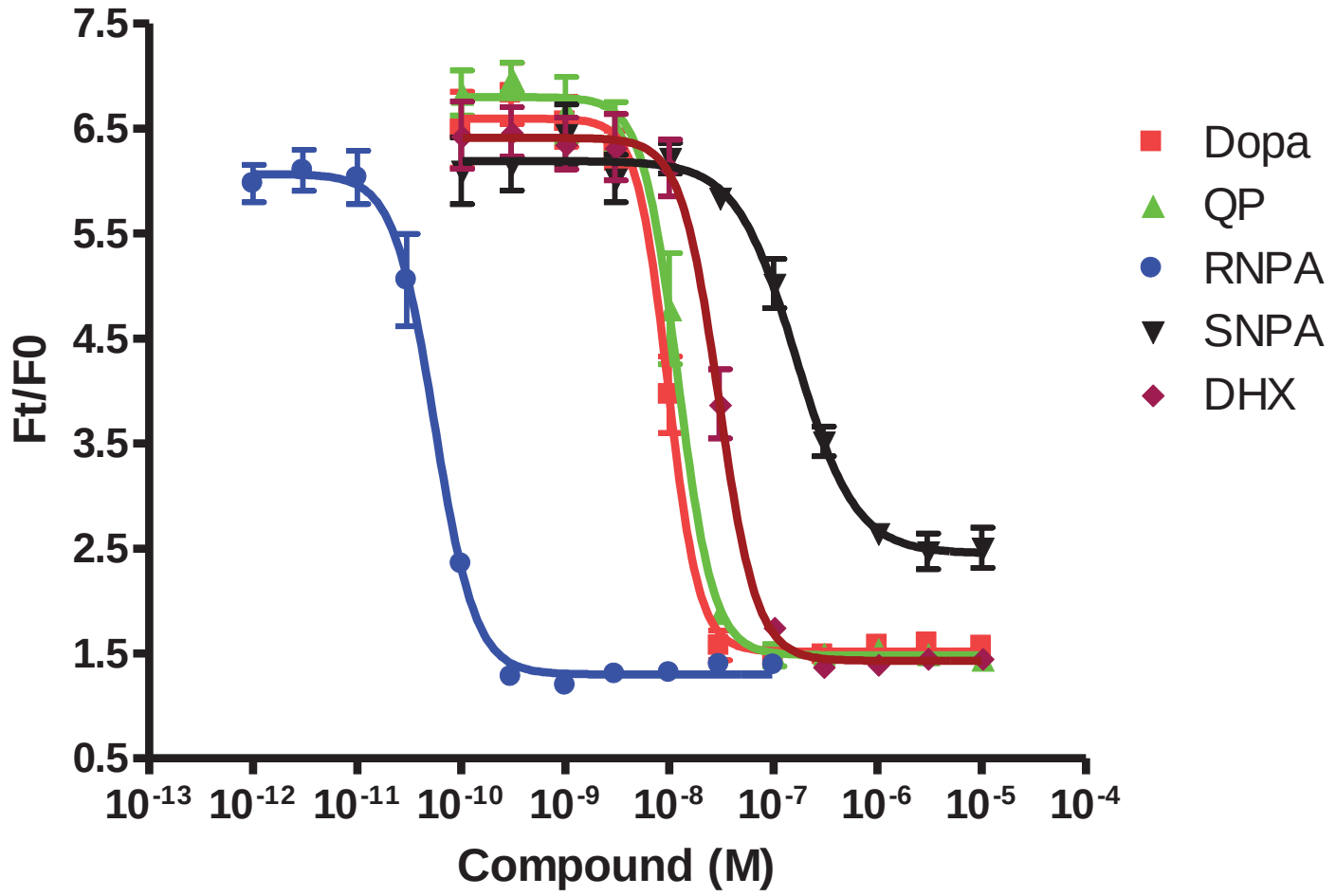
Dopamine Inhibits Forskolin Induced cAMP Activation in DRD2 Stable Cells (Kinetic Assay)



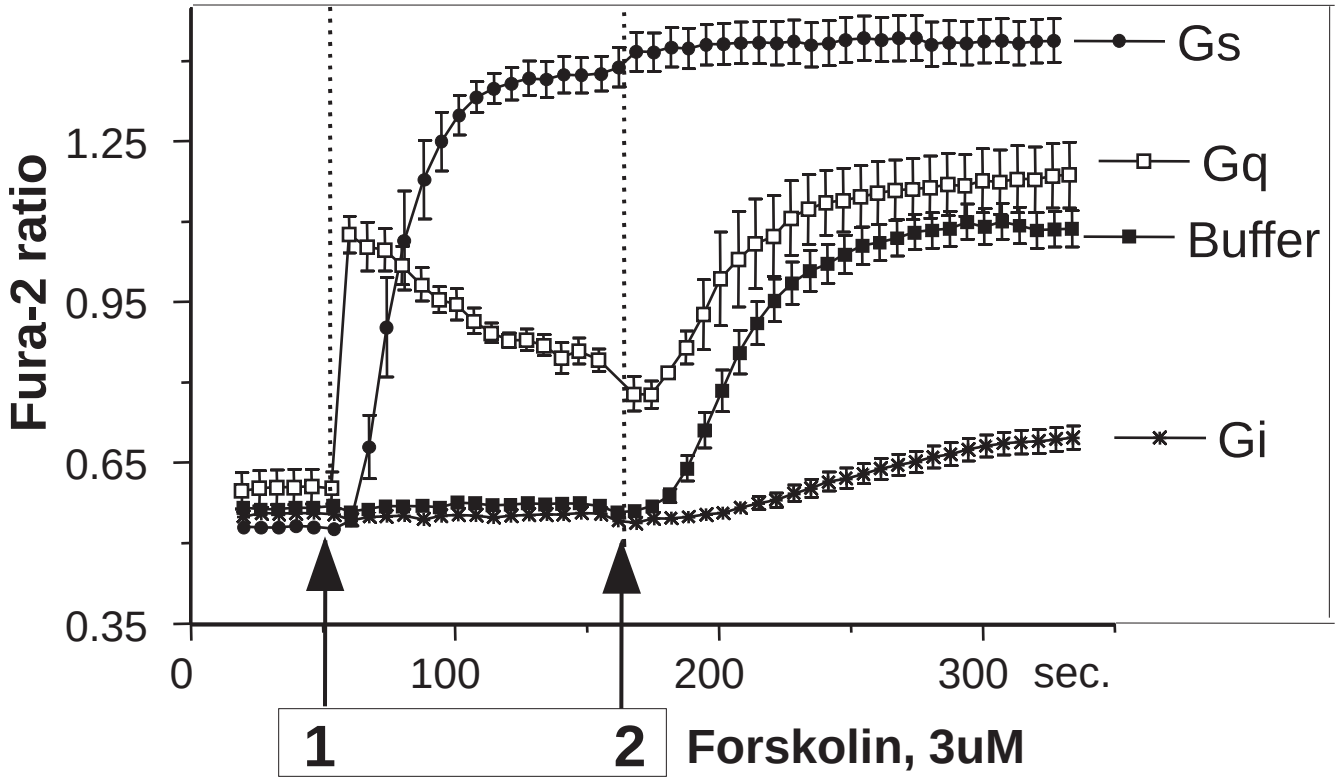
Endpoint Assay for Gi-Coupled DRD2



ACTOne Detects Partial Agonist of DRD2



ACTOne Differentiates Gq, Gs and Gi Pathway



Gs, Isoproterenol 1uM

Gq, Carbachol 10uM

Gi, Dopamine 300nM

Buffer

List of ACTOne Stable Clones

Transduced Gi-coupled receptors (22)

- Cannabinoid receptor 1 (CB1)
- Dopamine Receptor 2 (DRD2)
- Somatostatin Receptor 5 (SSTR5)
- Adenosine A1 Receptor (ADORA1)
- Chemokine (C-C motif) receptor 5 (CCR5)
- Melanin-concentrating Hormone Receptor 1 (MCHR1)
- Cannabinoid receptor 2 (CB2)
- Glutamate receptor, metabotropic 8 (GRM8)
- Opioid receptor, kappa 1 (OPRK1)
- Adenosine A3 receptor (ADORA3)
- Glutamate receptor, metabotropic 8 (GRM8)
- Neuropeptide Y Receptor Y1 (NPY1R)
- Neuropeptide Y Receptor Y2 (NPY2R)
- Sphingosine-1-phosphate receptor 1 (EDG1)
- Glutamate receptor, metabotropic 7 (GRM7)
- Glutamate receptor, metabotropic 2 (GRM2)
- Chemokine (C-X-C motif) receptor 4 (CXCR4)
- Opiate receptor-like 1 (OPRL1)
- Angiotensin II receptor-like 1 (AGTRL1)
- Opioid receptor, mu 1 (OPRM1)
- Glutamate receptor, metabotropic 4 (GRM4)
- Neuropeptide Y Receptor Y4 (NPY4R)

Transduced Gs coupled receptors (34)

- Vasoactive Intestinal Peptide Receptor 2 (VIPR2)
- Melanocortin 4 Receptor (MC4R)
- Melanocortin 5 Receptor (MC5R)
- Parathyroid Hormone Receptor 1 (PTHR1)
- Glucagon Receptor (GCGR)
- Dopamine Receptor 1 (DRD1)
- Prostaglandin E Receptor 4 (EP4)
- Vasoactive Intestinal Peptide Receptor 1 (VIPR1)
- Gastric Inhibitor Peptide Receptor (GIPR)
- Dopamine Receptor 5 (DRD5)
- Parathyroid Hormone Receptor 2 (PTHR2)
- 5-hydroxytryptamine (serotonin) receptor 6 (HTR4)
- Corticotropin Releasing Hormone Receptor 2 (CRHR2)
- Adenylate Cyclase Activating Polypeptide 1 Receptor type I (ADCYAP1R1)
- Secretin Receptor (SCTR)
- Follicle Stimulating Hormone Receptor (FSHR)
- 5-hydroxytryptamine (serotonin) receptor 6 (HTR6)
- 5-hydroxytryptamine (serotonin) receptor 7 (HTR7B)
- Glucagon-like peptide 2 receptor (GLP2R)
- Thyroid stimulating hormone receptor (TSHR)
- Melanocortin 3 Receptor (MC3R)
- Arginine Vasopressin Receptor 2 (AVPR2)
- Prostaglandin I2 Receptor (PTGIR)
- Glucagon-like-1 Receptor (GLPR1)
- Corticotropin Releasing Hormone Receptor 1 (CRHR2)
- Calcitonin receptor-like (CALCRL)
- Adenosine A2b receptor (ADORA2B)
- Beta2 Andrenergic Receptor (ADRB2)
- Prostaglandin D2 receptor (PTGDR)
- Prostaglandin E receptor 2 (PTGER2)
- Melanocortin 1 Receptor (MC1R)
- Calcitonin receptor (CALCR)
- Adenosine A2a receptor (ADORA2a)
- Amylin 3 receptor (Amy3R)

Summary of ACTOne on GPCRS

- **Measures physiological levels of cAMP in living cells**
- **Excellent signal to noise ratio and reproducibility**
- **Fast Response and stable signal allowing kinetic or endpoint assay formats**
- **Screen agonists and antagonists in one assay.**
- **Advantage to detect endogenous GPCRs**
- **Powerful deorphanizing tool to help study novel receptors**