

LANCE™ cAMP – Increased Sensitivity, Stability and Overall Assay Performance with G_{αs} and G_{αi} Coupled Receptors in a Miniaturized 1536-Well Format.

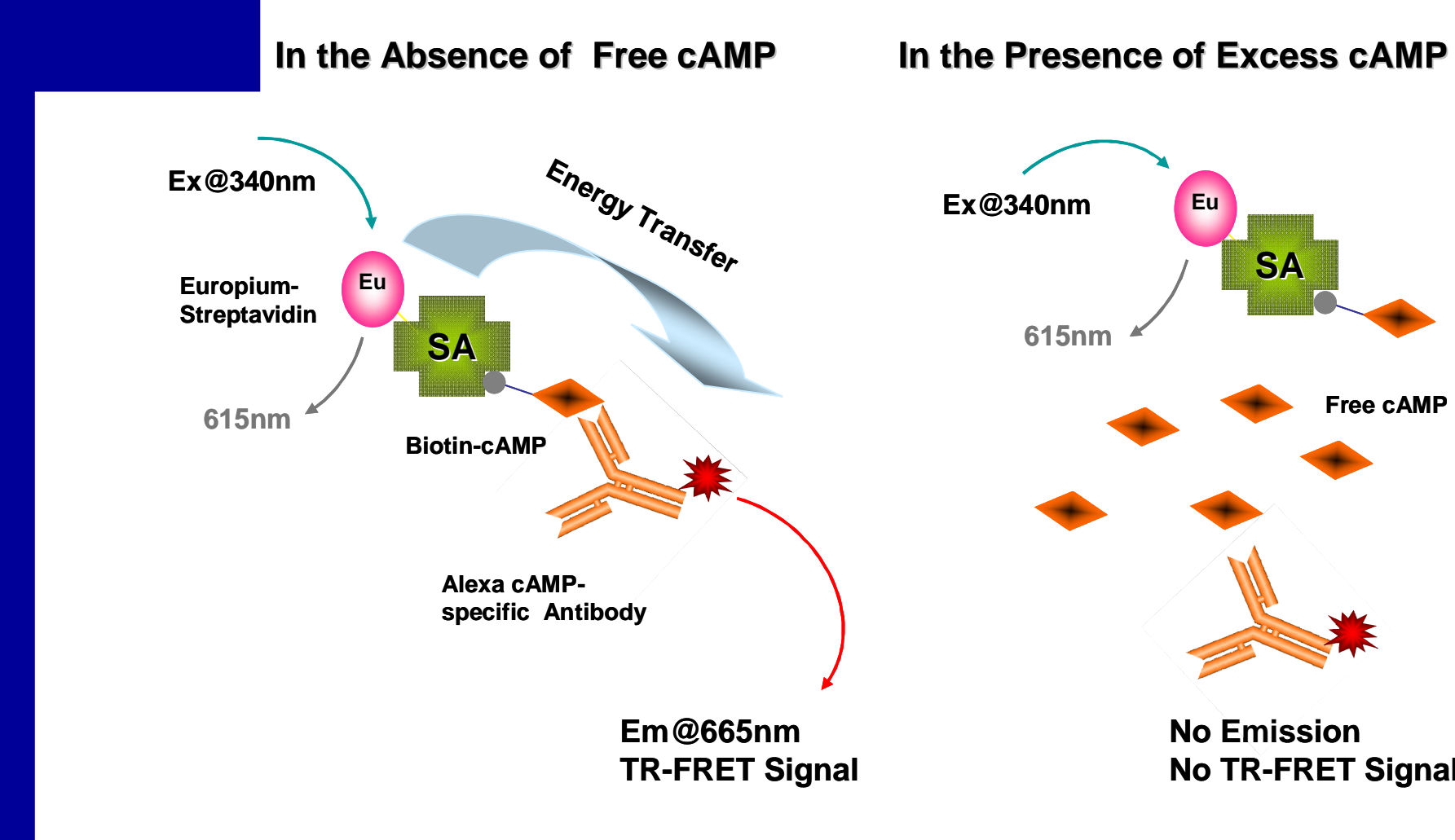
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1 Abstract

Time resolved fluorescence (TRF) assays exhibit low background and high signal-to-noise ratios, two attributes critical for robust HTS assays. LANCE™ refers to homogeneous TRF applications using TR-FRET. In the previous version of the LANCE cAMP assay, energy was transferred from europium labeled antibody to an acceptor, allophycocyanin (APC) labeled to cAMP. The assay has now been improved by using an antibody labeled with the acceptor, Alexa Fluor® 647 which significantly improved assay sensitivity, stability and overall performance. The principle of the assay involves the loss of energy transfer as the quantity of cell derived cAMP increases, thereby competing and therefore decreasing the amount of biotinylated-cAMP/streptavidin europium available to bind to the Alexa dye labeled anti-cAMP antibody. Due to the format change, the assay has now been proven to provide superior performance with G_{αs} and G_{αi} whole cell cAMP assays in 1536-well formats.

2 Schematic Representation of LANCE cAMP Assay



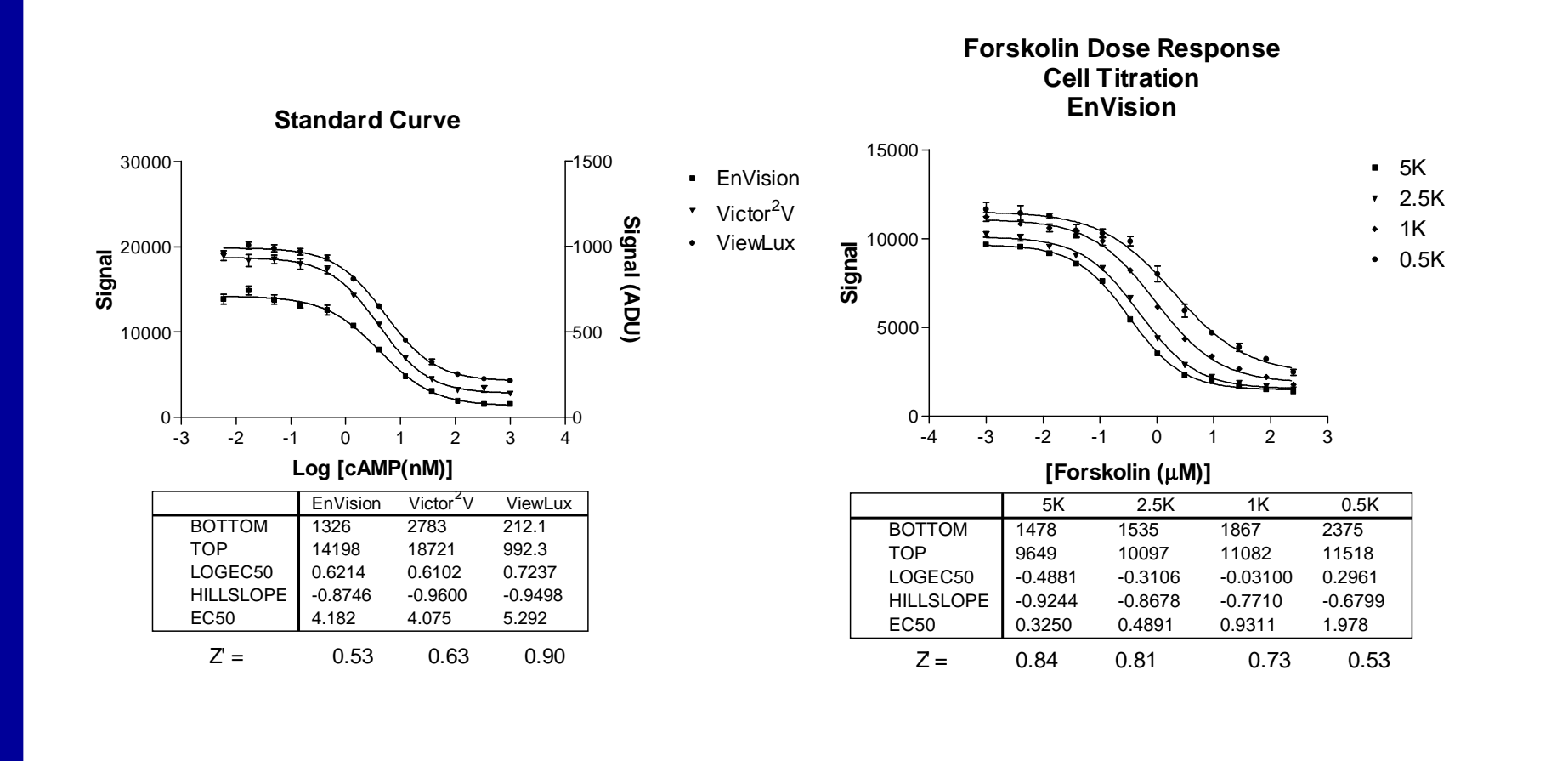
3 Materials

- > LANCE cAMP Assay (PerkinElmer)
 - The kit includes the following components:
 - Biotinylated- cAMP
 - Streptavidin Europium
 - Alexa Labeled Antibody
 - cAMP Standard
 - Detection Buffer
- > OptiPlate (1536-well white plate) (PerkinElmer Catalog # 6005228)
- > Cell line - β2 adrenergic receptor cell line (PerkinElmer MCL-501) and 5HT1A receptor cell line (PerkinElmer MCL-508)
- > Chemicals-Forskolin (Sigma# 6886)
- Agonists (Epinephrine Sigma# E4250), (8OH-DPAT Sigma# H140)
- Antagonists(Propranolol Sigma# P-0884) (Spiperone Sigma# S-7395)

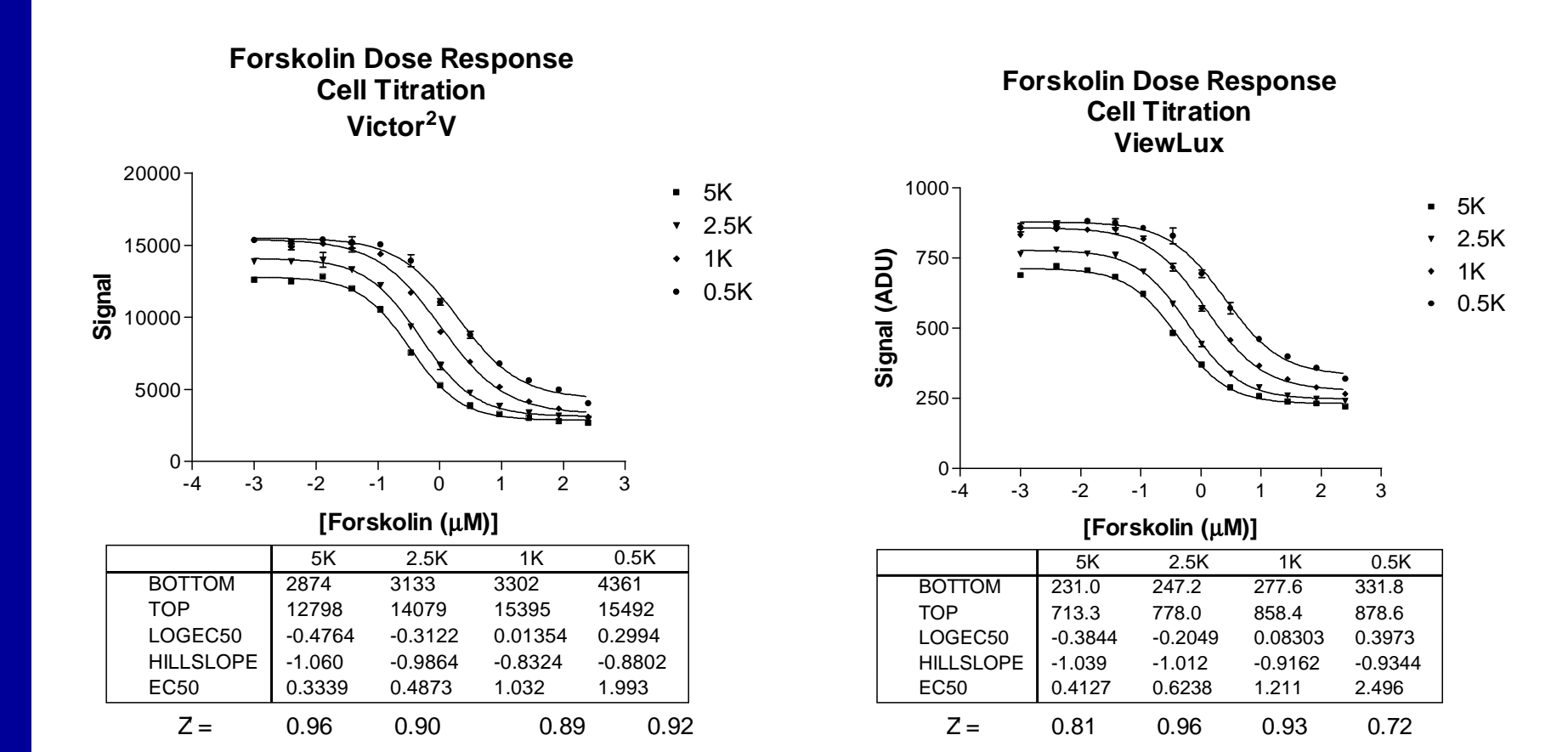
4 Methods

- > LANCE™ cAMP Assay
 - 2 μL of cAMP standards, forskolin, agonist, antagonist in PBS
 - 2 μL cells diluted in Stimulation Buffer containing cAMP antibody or Stimulation Buffer containing cAMP antibody without cells added to the wells containing standards
 - Incubate for 30 – 60 minutes at room temperature
 - 4 μL of Detection Buffer containing Biotin-cAMP and SA-Eu
 - Incubate for 60 minutes at room temperature
 - Read on an appropriate reader

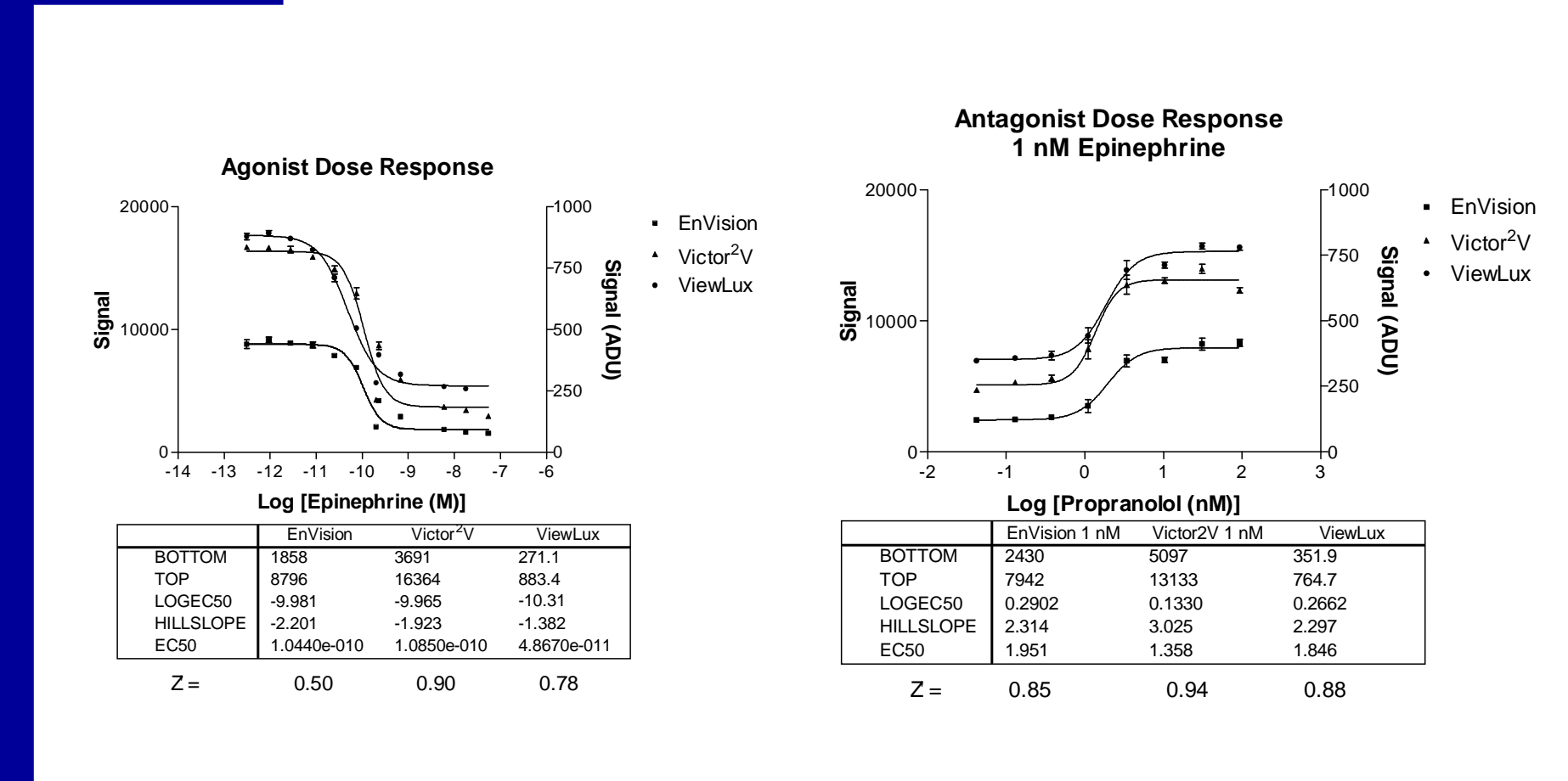
5 Results β2 Adrenergic Receptor



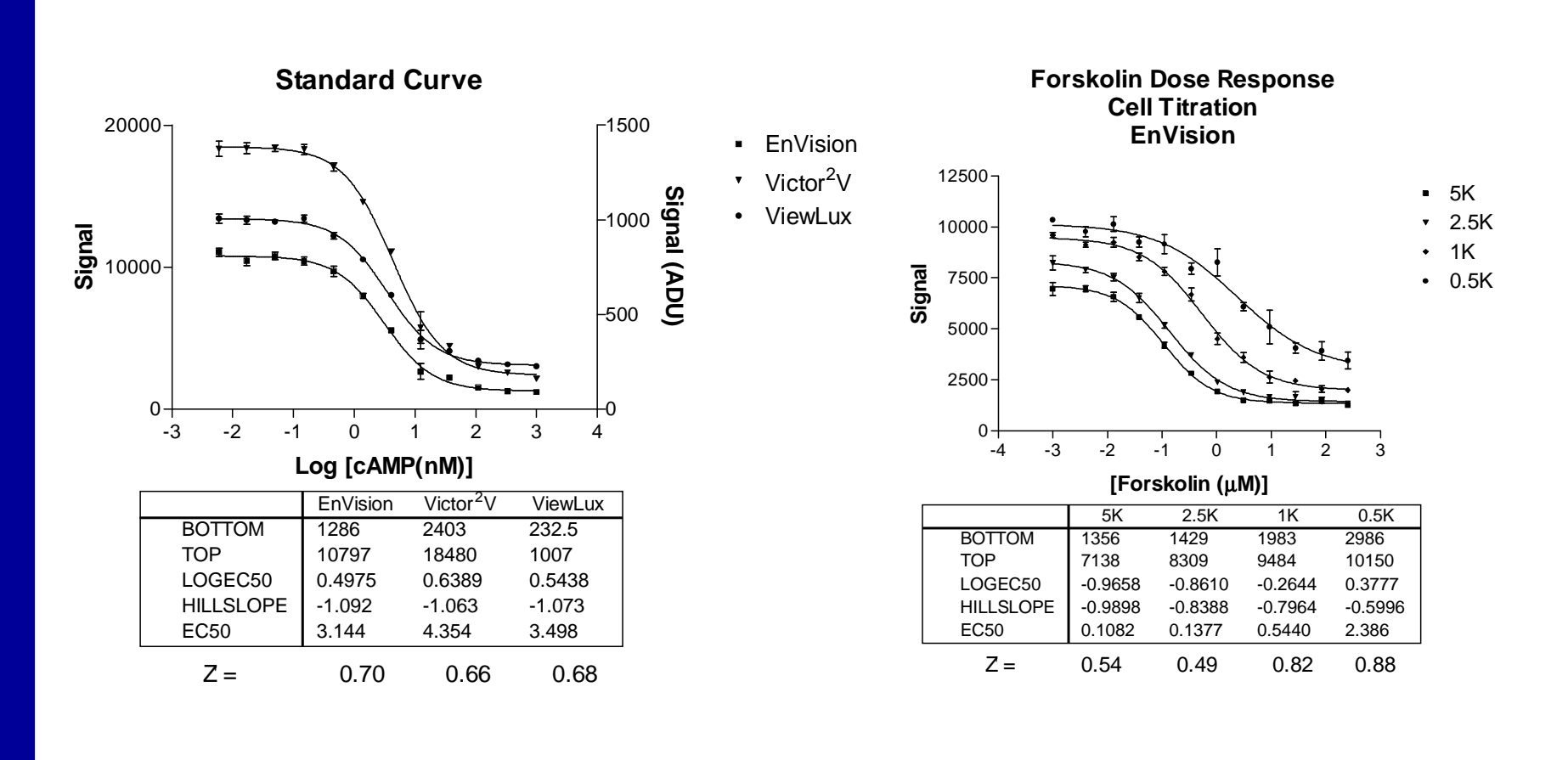
6 Results β2 Adrenergic Receptor



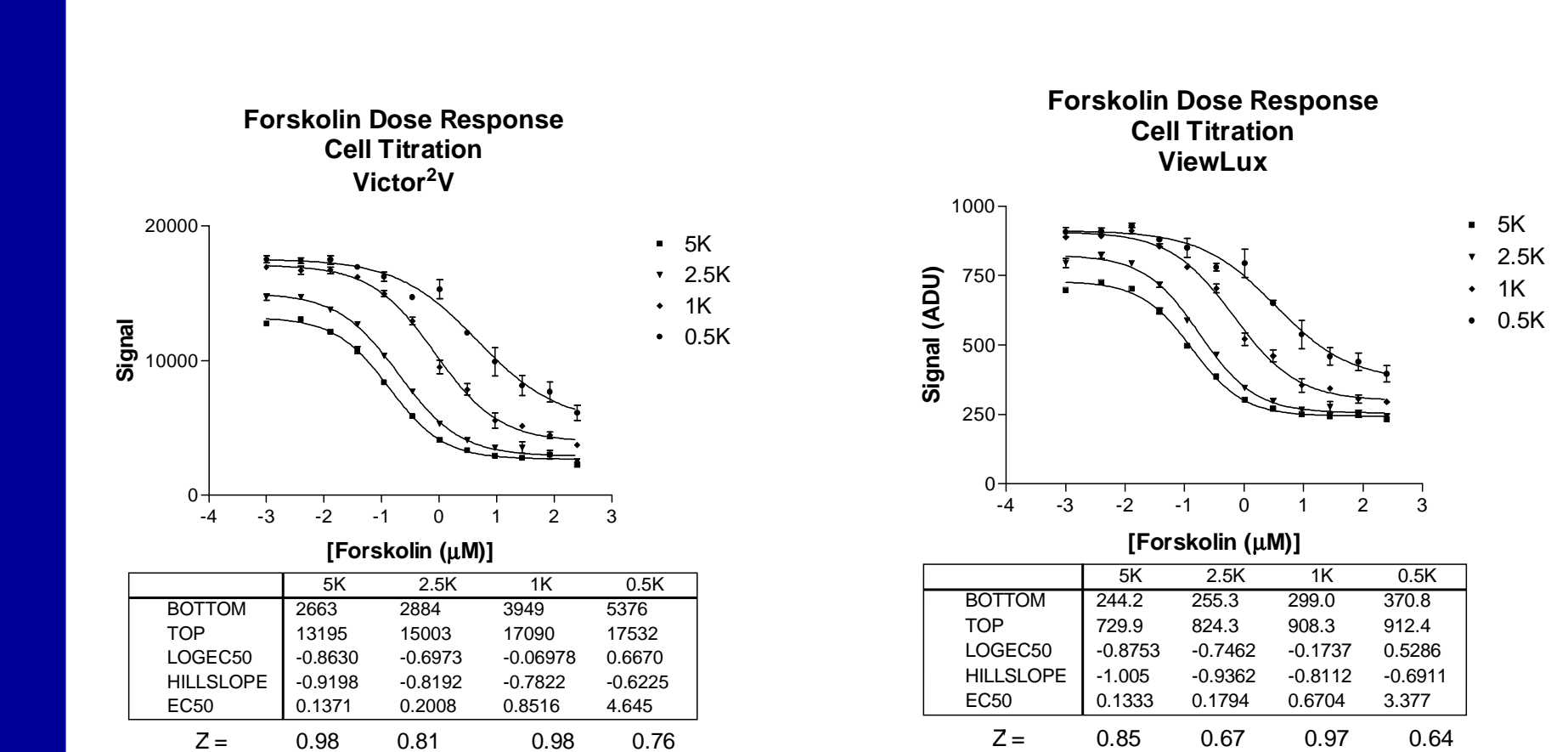
7 Results β2 Adrenergic Receptor



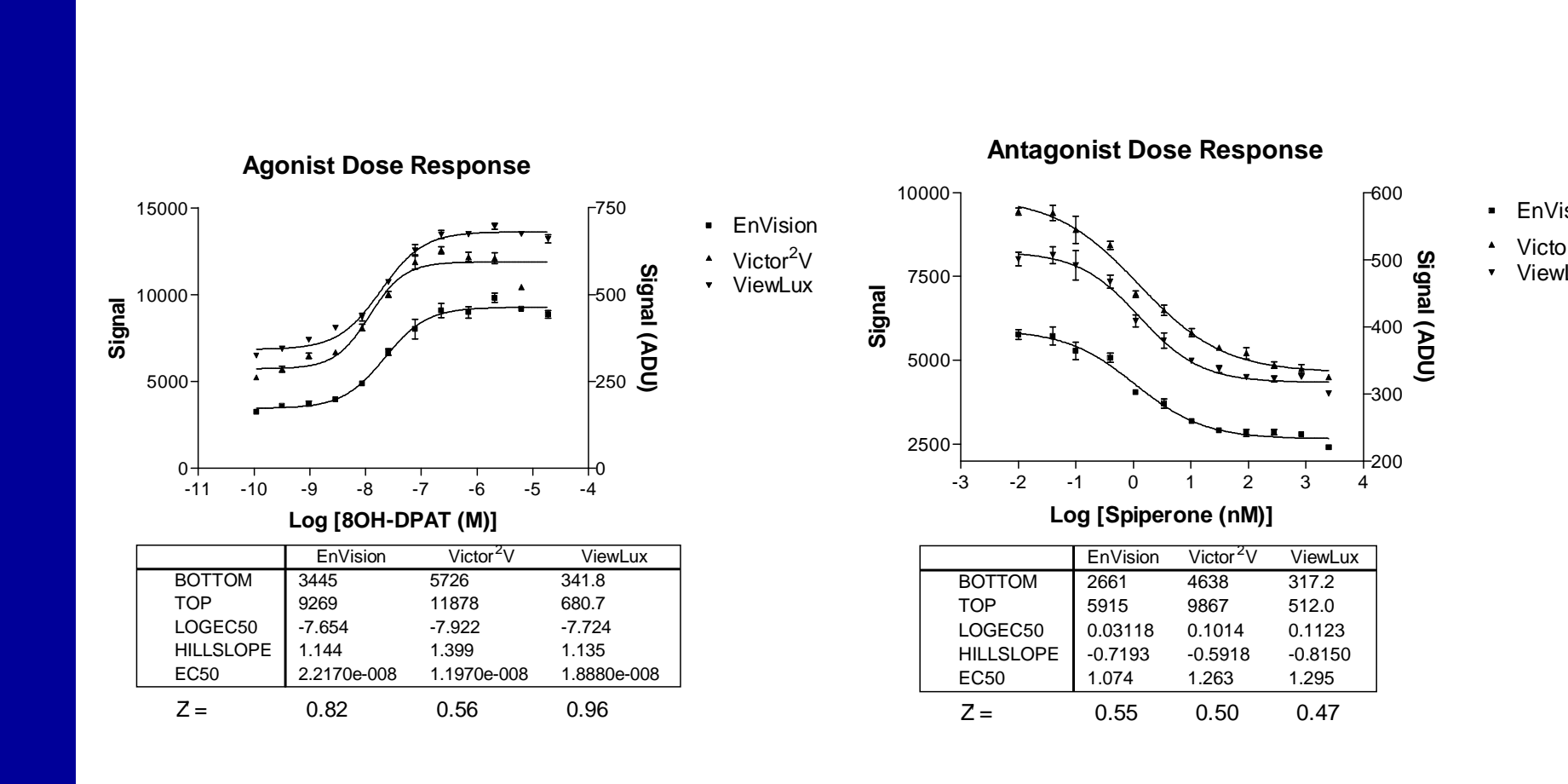
8 Results 5HT1A



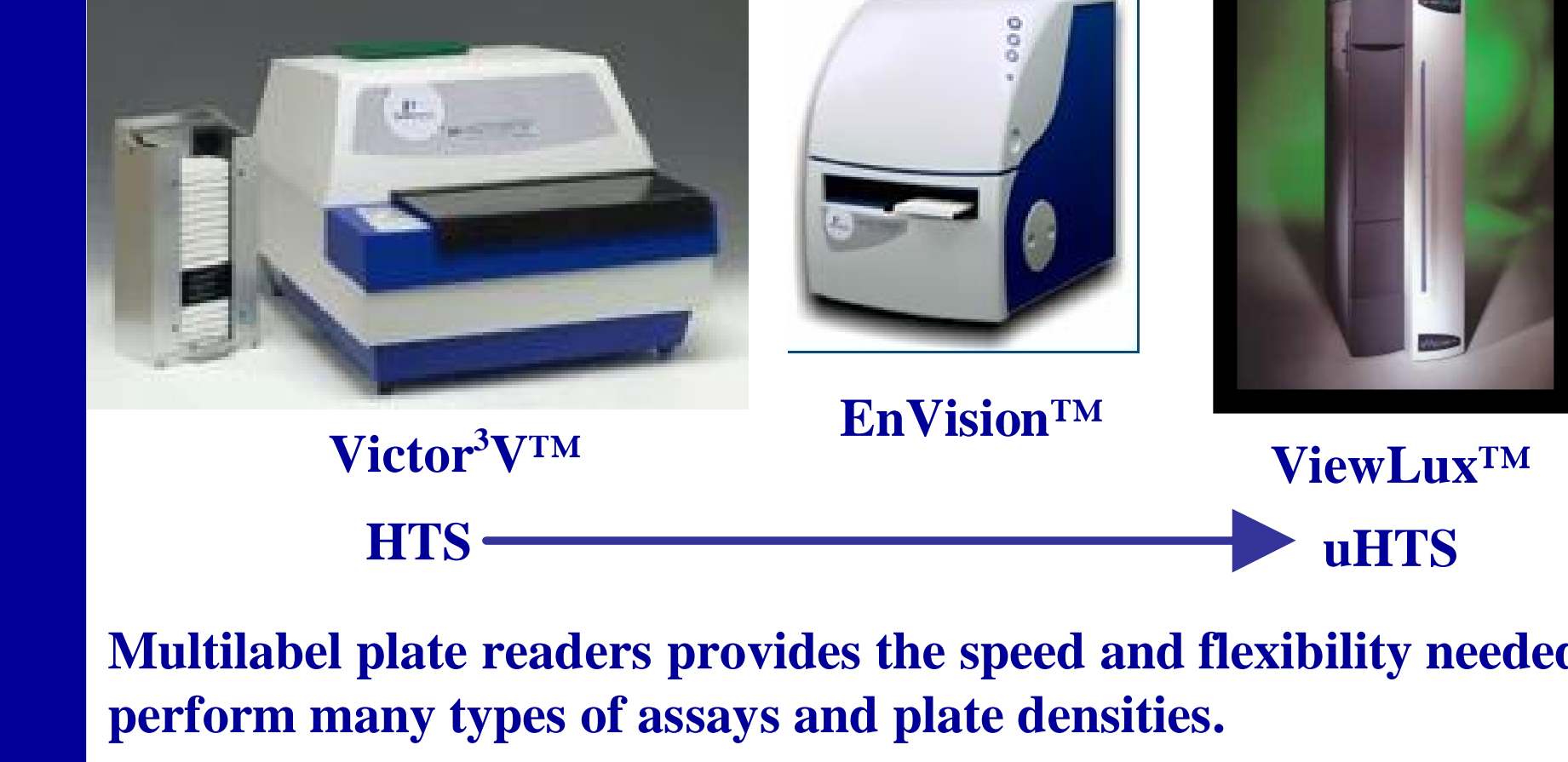
9 Results 5HT1A



10 Results 5HT1A



11 Detection Instruments



12 Conclusion

- > Excellent performance in a 1536-well format
- > Excellent performance of both G_{αs} and G_{αi} coupled receptors
- > Correct pharmacology as compared to literature values
- > Dramatic cost savings
 - Requires less reagents/well
 - Requires less cells/well
- > Ultra High Throughput Screening capability
- > Simple protocol
- > Easily adapted to robotic systems