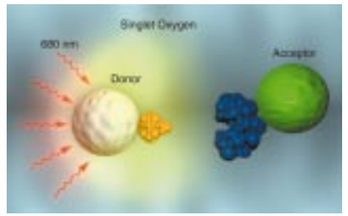
AlphaScreen™ to Measure cAMP induction with SignalScreen™ Dopamine D1 receptor membranes

Roger Bossé, Lucille Beaudet, Martin Boissonneault, Nathalie Bouchard, Betty Howard**, Chantal Illy, Liliana Pedro, Geneviève Pinard, Philippe Roby, CJ Wang* & Daniel Chelsky. BioSignal Packard *Packard Bioscience Meriden Connecticut, **Packard Bioscience Downers Grove

ABSTRACT

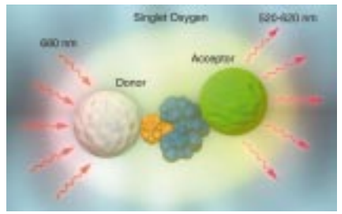
AlphaScreen is a novel, homogeneous, nonradioactive assay technology applicable to a broad range of HTS and assay development applications. Based on the proximity of two very small beads and an amplified luminescence signal, AlphaScreen results in a very intense signal output with robust signal/background ratios. These unique characteristics allow the technology to be applied to small volume assays without changing assay component concentrations. Off the shelf beads are available with a large variety of coatings to make a wide range of assay types possible. Examples of the assay types currently validated include serine/threonine kinases, tyrosine kinases, proteases, DNA helicase, functional cAMP, and a variety of ligand/receptor binding, protein protein interactions, transcription factor/DNA, and low affinity binding (1 uM) interactions. The AlphaQuest HTS Microplate Analyzer with a 40-plate stacker and internal bar code reader allows for rapid processing of assay plates regardless of sample density, 96, 384 or 1536. In this poster we will demonstrate the ability to perform cAMP induction or inhibition studies with SignalScreen L cell membranes available from BioSignal Packard. Combined with Alpha Screen technology, this provides a rapid , homogeneous, sensitive assay without the variability or time consuming preparation of whole cells. Assays can be run on demand without waiting for the preparation of cells; membranes are stored frozen and can be used whenever needed. Provided here is data using the Dopamine D1 receptor as the membrane preparation and the comparable data expressed in whole cells.

Figure 1. Principles of AlphaScreen



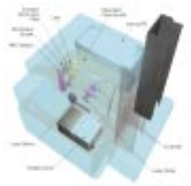
Laser irradiation of the Donor beads at 680 nm generates a flow of short-lived singlet oxygen molecules. When the Acceptor beads are not in proximity, the reactive oxygen decays and there is no signal.

Figure 2. Principles of Alpha Screen [continued]



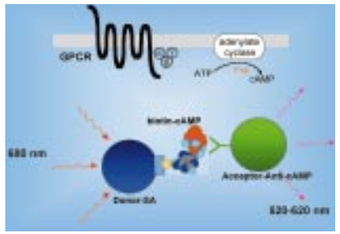
When biological interactions bring the Donor and Acceptor beads into close proximity, reactive oxygen, generated by irradiation of the Donor beads, initiates a luminescence/fluorescence cascade in the Acceptor beads. This process leads to a highly amplified signal with output in the 520-620 nm range.

Figure 3. AlphaQuest HTS Microplate Analyzer



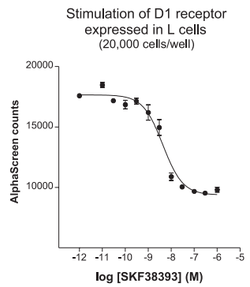
The AlphaQuest-HTS is a four-detector instrument, optimized for AlphaScreen chemistry, using highly efficient diode excitation at 680 nm with high performance optics detecting emission at 520-620 nm. With a 40-plate stacker and bar code reader, the AlphaQuest-HTS reads 96-well plates in under 1 minute, 384-well plates in 2.5 minutes and 1536-well plates in 8.5 minutes.

Figure 4. Principles of cAMP with AlphaScreen



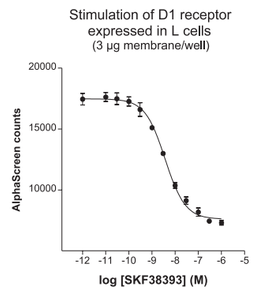
AlphaScreen cAMP has been designed to directly measure levels of cAMP produced upon modulation of adenylate cyclase activity by G-protein coupled receptors. AlphaScreen cAMP is based on the competition between endogenous cAMP and exogenously added biotin-cAMP. The capture of cAMP is achieved by using a specific antibody conjugated to Acceptor beads. The assay is efficient at measuring both agonist and antagonist activities on Gi and Gs coupled GPCRs. AlphaScreen cAMP is specific and reliable. This assay is highly competitive with existing cAMP assays in terms of ease of use, sensitivity, dynamic range and time to completion.

Figure 5. SKF38393 induced cAMP production by L cells expressing D1 receptors



Protocol as per Technical Note on Functional cAMP assays from BioSignal Packard or Packard BioSciences. Application Note also available at www.packardinstrument.com.

Figure 6. SKF38393 induced cAMP production by L cell membrane preparation expressing D1 receptors



Membrane Assay protocol

Buffer 1: Oligo element buffer: 25 mM MgCl2, 375 mM NaCl2, 250 uM ATP, 2.5 µM GDP, 2.5 nM GTP. All dissolved in water.

Buffer 2: Lysis buffer (also used for cell based assay): 5 mM Hepes, pH 7.4, 0.1% BSA, 0.3% Tween 20.

Assay Protocol:

10 µl of cAMP acceptor beads (15 µg/ml final concentration in oligo element buffer)
10 µl membranes (3 µg/well, product # 6100526)
5 µl SKF38393 (forskolin, or unlabeled cAMP for standard curves would be added at this amount)
Incubate 30 minutes at Room temperature.
Add 25 µl of Streptavidin donor beads (20 µg/ml final in lysis buffer), premixed with biotin cAMP (10 nM final concentration)
Incubate 1 hour at Room temperature.
Read on AlphaQuest.

Results

	S/B	IC_{50}
Cells	1.9	4.3 nM
Membranes	2.3	3.7 nM

Summary

The use of SignalScreen membranes with AlphaScreen reagents, combined with the rapid reading time of the AlphaQuest Microplate Analyzer make this assay ideal for High Throughput cAMP assays. The data represented here shows comparable results between membrane preparations and the equivalent cell based assay and values commonly reported in the literature. As the buffers required for the membrane assay are optimized, to mimic the cell condition, the assay can be done on demand. Membranes can be stored frozen and used on any day of the week or month as time permits. They are not subject to the changes or variation seen in cell culture requiring assays. The current list of SignalScreen membranes expressing GPCR's, as well as a complete list of currently available AlphaScreen reagents can be obtained from Packard BioSciences.

BioSignal Packard Inc., 1744 William St., Suite 600, Montreal (Quebec), H3J 1R4
Packard Instrument Company, 800 Research Parkway, Meriden, Connecticut 06450