ALPHASCREEN™ to Measure cAMP induction with SIGNALSCREEN™

Dopamine D1 receptor membranes


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INTRODUCTION

AlphaScreen is a versatile, homogeneous, nonradioactive assay technology applicable to a broad range of HTS and assay development applications. The AlphaScreen technology is based on the principle that two very small, non-fluorescent solid support particles, referred to as the Donor and Acceptor beads, are used in conjunction with the luminescence signal. AlphaScreen uses a very minute amount of energy to initiate signal generation. These unique characteristics allow the technology to be applied in small volume assays without changing assay component concentrations. For the assay, each bead is coated with a particular biotinylated ligand. AlphaScreen is a proprietary technology that includes a dual-signal strategy to initiate luminescence generation. The dual-signal strategy uses a close proximity, reactive oxygen, generated by irradiation of the Donor beads, to induce a luminescence/fluorescence cascade in the Acceptor beads. The process leads to a highly amplified signal output in the 520-620 nm range.

ALPHAQUEST HTS Microplate Analyzer is an automated, a variety of ligand/receptor binding, protein-protein interactions, kinase, tyrosine kinase, protease, DNA helicase, functional cAMP, and a variety of coatings to make a wide range of assay types possible. Off the shelf beads are available with robust signal/background ratios. These unique characteristics allow the technology to be applied in small volume assays without changing assay component concentrations. For the assay, each bead is coated with a particular biotinylated ligand. AlphaScreen uses a very minute amount of energy to initiate signal generation. These unique characteristics allow the technology to be applied in small volume assays without changing assay component concentrations. For the assay, each bead is coated with a particular biotinylated ligand.

Carbonic anhydrase is a membrane-associated enzyme that catalyzes the hydration of carbon dioxide to form bicarbonate ions. This reaction is a critical step in the production of bicarbonate, which is then transported to the mitochondria for conversion to ATP. ATP production is essential for cellular energy metabolism and is a key process in the function of many cell types. Carbonic anhydrase is expressed in various tissues, including the heart, lung, kidney, and brain. The enzyme helps maintain the acid-base balance in the body, which is crucial for proper cellular function.

RESULTS

Membrane Assay protocol

Buffer 1: Lysis buffer: 5 mM Hepes, pH 7.4, 100 mM NaCl, 0.1% Triton X-100

Buffer 2: Oligo element buffer: 25 mM MgCl₂, 375 mM NaCl₂, 250 uM ATP, 2.5 µM GTP, 2.5 mM GDP, 2.5 nM GTP. All dissolved in water

Assay Protocol:

1. Membranes 2.3 ± 0.7 nM

2. Cells 1.9 ± 4.3 nM

Membrane Activity

The AlphaScreen cAMP assay can be used to measure cAMP levels in a variety of cellular preparations, including cell membranes, cell lysates, and intact cells. The assay is based on the competitive binding of endogenous cAMP and an exogenous biotinylated cAMP substrate to streptavidin-coated donor beads. When the donor and acceptor beads are brought into close proximity, the energy from the donor beads is transferred to the acceptor beads, generating a luminescence signal. The signal is proportional to the concentration of cAMP in the sample.

Figure 1. Principles of AlphaScreen

Figure 2. Principles of AlphaScreen (continued)

Figure 3. Membrane Assay protocol

Figure 4. Principles of cAMP with AlphaScreen

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 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.

Figure 5. Q538355 induced cAMP production by L cell expressing D1 receptors

Figure 6. Q538355 induced cAMP production by L cell membrane expressing D1 receptors

Figure 7. SKF38393 induced cAMP production by L cell expressing D1 receptors

Figure 8. SKF38393 induced cAMP production by L cell membrane expressing D1 receptors

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