AlphaScreen™ True format flexibility for 96, 384 or 1536 well formats.

BioSignal Packard
A Packard BioScience Company

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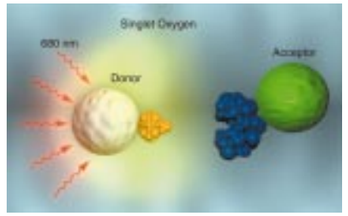
ABSTRACT

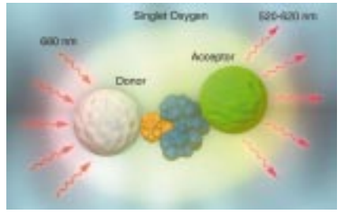
AlphaScreen is a novel, homogeneous, nonradioactive assay technology applicable to a broad range of HTS and assay development applications. Based on the proximity of two very small beads and an amplified luminescence signal, AlphaScreen results in a very intense signal output with robust signal/background ratios. These unique characteristics allow the technology to be applied to very 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 µM) 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 migrate to the desired or needed plate density with this single technology. Using cAMP as an example, we will show data with the same reagent concentrations in all three formats. The choice is yours.

Figure 1. Principles of AlphaScreen



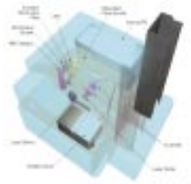
Laser irradiation of the Donor beads at 680 nm generates a flow of short-lived singlet oxygen molecules. When the Acceptor beads are not in proximity, the reactive oxygen decays and there is no signal.

Figure 2. Principles of Alpha Screen [continued]



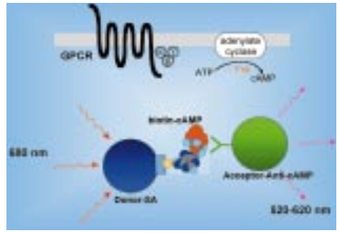
When biological interactions bring the Donor and Acceptor beads into close proximity, reactive oxygen, generated by irradiation of the Donor beads, initiates a luminescence/fluorescence cascade in the Acceptor beads. This process leads to a highly amplified signal with output in the 520-620 nm range.

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 6. Cell-Based GPCR Functional Assay for cAMP



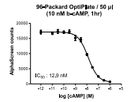
AlphaScreen cAMP has been designed to directly measure levels of cAMP produced upon modulation of adenylate cyclase activity by G-protein coupled receptors. AlphaScreen cAMP is based on the competition between endogenous cAMP and exogenously added biotin-cAMP. The capture of cAMP is achieved by using 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.

cAMP Standard Curve at 1 hour assay incubation

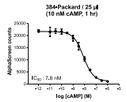
Protocol:

Anti cAMP Acceptor beads at 15µg/ml, Streptavidin Donor beads at 20 µg/ml, and biotin cAMP at 10 nM, final concentrations. Final assay volumes as indicated on each graph. Incubation 1 hour at room temperature. Plates were all read on AlphaQuest HTS for 1 second per well.

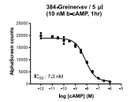
96 well Packard Optiplate.



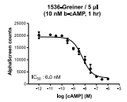
384 well Packard Optiplate



384 well Greiner Shallow well plate.



1536 Greiner plate.

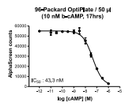


cAMP Standard Curve for 17 hour incubation

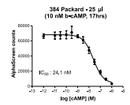
Protocol:

Anti cAMP Acceptor beads at 15µg/ml, Streptavidin Donor beads at 20 µg/ml, and biotin cAMP at 10 nM, final concentrations. Assay volumes as indicated on each graph. Incubation 17 hours at room temperature. Plates were all read on AlphaQuest HTS for 1 second per well.

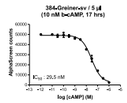
96 well Packard Optiplate.



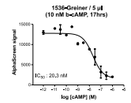
384 well Packard Optiplate



384 well Greiner Shallow well plate



1536 Greiner plate



Signal to Background and IC_{50} values

Table 1. 1 Hour assay

Plate	S/B	IC_{50} (nM)
Packard Optiplate 96	34	12.9
Packard 384	18.3	7.8
Greiner sv 384	17.6	7.3
Greiner 1536	7.8	6.0

Table 2. 17 hour incubation

Plate	S/B	IC_{50} (nM)
Packard Optiplate 96	56.8	43.3
Packard 384	17.5	24.1
Greiner sv 384	22.9	29.5
Greiner 1536	7.8	20.3

Summary

The light output and ease of use of AlphaScreen reagents, combined with the rapid reading time of the AlphaQuest reader make it ideal for assays regardless of format. The plate density choice will need to reflect your liquid handling capabilities (we use Packard CCS Plate Trak for low volume liquid handling), but is not limited by the amount of signal that can be produced. You can now use the same technology as you move to higher density without the need to change the assay dramatically or chose another technology. Data shown here gives assays with comparable results regardless of the plate density chosen. The reagents used to produce this data are packaged in per well units based on a 384 well assay in 25 µl total volume. In the case of the 96 well data, we have thus used more material per well than the packaged unit and with 1536 much less. We also have successfully worked in 96 wells at the reagent concentration of 384 well with similar results but requiring the 17-hour incubation (data not shown).

Conclusions

AlphaScreen is provides a homogeneous, nonradioactive technology that can work in 96, 384, and 1536 with the same reagents, at the same concentrations and read on the same plate reader. This technology has a wide range of possible applications for HTS or development needs.

AlphaScreen Reagents Currently available

- cAMP detection reagents
- Phosphotyrosine detection with PT66
- Phosphotyrosine detection with PY20
- GST tag detection
- HIS tag detection
- c-myc tag detection
- HA peptide detection
- Fluorescein detection
- Mouse IgG detection
- Rabbit IgG detection
- Human IgG detection
- Goat IgG detection
- FLAG detection
- Digoxin/digoxigenin detection
- Protein A

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