

Application Note

Principles of AlphaScreen™

Amplified Luminescent Proximity Homogenous Assay

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Introduction

AlphaScreen is a bead-based, non-radioactive **A**mplified **L**uminescent **P**roximity **H**omogeneous **A**ssay. When a biological interaction brings the beads together, a cascade of chemical reactions acts to produce a greatly amplified signal. On laser excitation, a photosensitizer in the “Donor” bead converts ambient oxygen to a more excited singlet state. The singlet state oxygen molecules diffuse across to react with a thioxene derivative in the Acceptor bead generating chemiluminescence at 370 nm that further activates fluorophores contained in the same bead. The fluorophores subsequently emit light at 520-620 nm (Figure 1).

In the absence of a specific biological interaction, the singlet state oxygen molecules produced by the Donor bead go undetected without the close proximity of the Acceptor bead. As a result, only a very low background signal is produced (Figure 2).

AlphaScreen provides a highly versatile, sensitive, homogeneous and miniaturizable means to efficiently perform assay development and HTS resulting in higher throughput at lower costs.

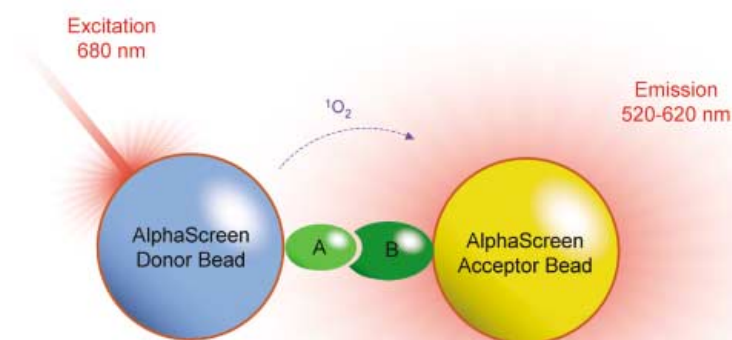


Figure 1: When biological interactions bring the Donor and Acceptor beads into close proximity, a highly amplified signal with output in the 520-620 nm range is generated.

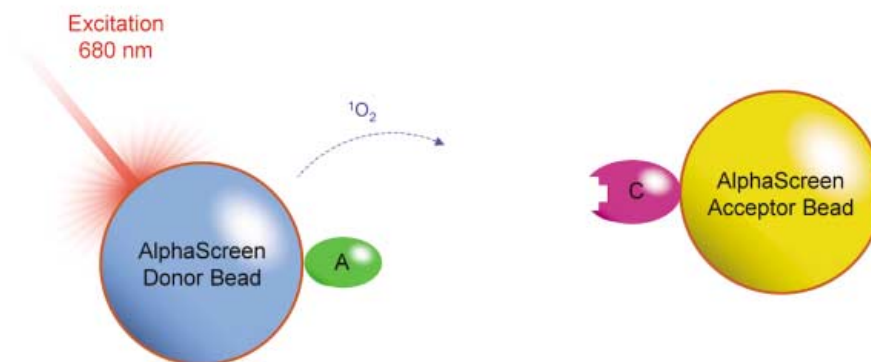


Figure 2: When the Donor and Acceptor beads are not in close proximity, the reactive oxygen decays and there is no detectable signal generated.

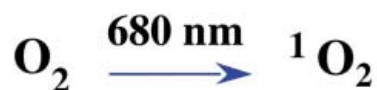
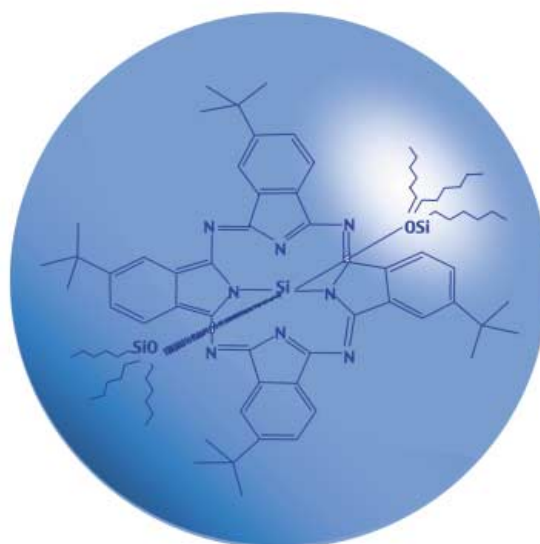


Figure 3: Oxygen to singlet oxygen conversion by the photosensitizer (phthalocyanine) in the Donor beads.

Principles of the Technology

The initial step in the AlphaScreen signal amplification procedure is the conversion of ambient oxygen to the singlet state by a photosensitizer in the Donor bead upon illumination at 680 nm (Figure 3). Because of the high concentration of photosensitizer, one Donor bead emits up to 60,000 singlet oxygen molecules per second. This results in very high signal amplification, allowing AlphaScreen assays to be miniaturized to very small volumes (< 5 μL) without increasing any assay reagent concentrations.

The Acceptor beads contain a thioxene derivative that reacts with the singlet oxygen to generate chemiluminescence at 370 nm. Energy transfer to fluorescent acceptors in the same beads shifts the emission wavelength to 520-620 nm. The half-life of the decay reaction is 0.3 sec, which makes the AlphaScreen fluorescence signal very long lived. The short lifetime of singlet oxygen in aqueous solution ($\sim 4 \mu\text{sec}$) allows diffusion over a distance up to $\sim 200 \text{ nm}$.

The AlphaScreen beads are coated with a layer of hydrogel which retains the dyes, minimizes non-specific binding and particle self-aggregation, and provides functional groups for bioconjugation. The size of the beads is optimized and uniform, they are small enough to prevent settling in aqueous suspensions and are easily dispensed using automated liquid handling, yet are large enough to be centrifuged. Additionally, because the illumination wavelength is very high at 680 nm, very few biological components and assay compounds will interfere.

AlphaScreen Signal Detection

The AlphaScreen signal is best read on either the Fusion™-α Multilabel Reader or the AlphaQuest®-HTS Microplate reader available from PerkinElmer Life Sciences.

Fusion-α

Fusion-α is a Multilabel Reader designed to measure top and bottom fluorescence intensity, time-resolved fluorescence, absorbance, luminescence and AlphaScreen technologies in a single instrument. Fusion-α can read samples in all microplate formats. The versatility of this system makes it an ideal tool to meet assay development requirements.

AlphaQuest-HTS

AlphaQuest-HTS is a dedicated 4-detector system to read AlphaScreen in all microplate formats. A1536-well plate can be read in less than 8 minutes. AlphaQuest-HTS is recommended for high throughput screening applications and can be integrated into robotic systems to streamline HTS operations.

Applications

AlphaScreen is successfully developed for and adapted in many assay formats, including:

- ▶ GPCR functional assays (cAMP and IP₃)
- ▶ Enzyme assays (tyrosine kinase, serine/threonine kinase, helicase, protease, phosphatase)
- ▶ Interaction assays (cytokine binding assays, nuclear receptor functional assays, ligand-receptor binding assays, protein/protein, protein/DNA, protein/peptide)
- ▶ ELISA conversion (immunoassays)
- ▶ Homogeneous Phage Display and some more novel applications

For more information regarding the applicability of AlphaScreen towards the development of your assay of interest, please contact our customer support center.

Features and Benefits of AlphaScreen

Homogeneous

- AlphaScreen is a true homogeneous assay that does not require separation steps or washes.

Sensitive

- An amplified AlphaScreen signal resulting from the 60,000 singlet oxygen molecules generated by each Donor bead allows detection down to the attomolar (10⁻¹⁸) level in some biological assays.

Low Background

- A long excitation wavelength of 680 nm combined with a shorter emission wavelength of 520-620 nm reduces interference from biological or assay components and ensures a very low background.

Easy to Use

- AlphaScreen is available in a variety of ready-to-use detection kits for pre-validated assays using off-the-shelf reagents.

Automatable

- AlphaScreen beads are very small (200 nm in diameter) and do not settle or clog pipette tips, thereby simplifying automated liquid handling requirements and are ideally suited for HTS.

Miniaturizable

- AlphaScreen is readily adaptable to 96-, 384- and 1536-well formats without changing reagent concentrations or compromising sensitivity.

Highly Versatile

- Enzymes, receptor-ligand interactions, low affinity interactions, second messenger levels, DNA, RNA, proteins, peptides, carbohydrates and small molecules can be assayed with AlphaScreen.

AlphaScreen Reagents and Kits

Reagents	Cat #	1 mg	5 mg	50 mg
• Unconjugated Acceptor beads		6762003	6762001	6762002
• Unconjugated Donor beads		6762013	6762011	6762012
• Streptavidin Donor beads		6760002S	6760002	6760002B

Fusion-Tag Detection Kits	Cat #	500 pts	10,000 pts	50,000 pts
• Glutathione-S-Transferase (GST) Detection Kit	6760603	6760603C	6760603M	6760603R
• Digoxin/Digoxigenin (Dig) Detection Kit	6760604	6760604C	6760604M	6760604R
• Fluorescein (FITC) Detection Kit	6760605	6760605C	6760605M	6760605R
• 6-Histidine (HIS ₆) Detection Kit	6760610	6760610C	6760610M	6760610R
• 6-Histidine (HIS ₆ -Nickel chelate) Detection Kit	6760619	6760619C	6760619M	6760619R
• c-myc Detection Kit	6760611	6760611C	6760611M	6760611R
• Hemagglutinin (HA) Detection Kit	6760612	6760612C	6760612M	6760612R
• FLAG™ Detection Kit	6760613	6760613C	6760613M	6760613R

IgG Detection Kits	Cat #	500 pts	10,000 pts	50,000 pts
• Mouse IgG Detection Kit	6760606	6760606C	6760606M	6760606R
• Goat IgG Detection Kit	6760608	6760608C	6760608M	6760608R
• Human IgG Detection Kit	6760609	6760609C	6760609M	6760609R
• Rabbit IgG Detection Kit	6760607	6760607C	6760607M	6760607R
• IgG (Protein A) Detection Kit	6760617	6760617C	6760617M	6760617R

GPCR Functional Assay Kits	Cat #	500 pts	10,000 pts	50,000 pts
• cAMP Assay Kit	6760600	6760600C	6760600M	6760600R
• IP ₃ Assay Supplement Kit (to be used with the GST Detection Kit)	6760621	6760621C	6760621M	6760621R

Phosphotyrosine Assay Kits	Cat #	500 pts	10,000 pts	50,000 pts
• Phosphotyrosine (P-Tyr-100) Assay Kit	6760601	6760601C	6760601M	6760601R
• Phosphotyrosine (PY20) Assay Kit	6760602	6760602C	6760602M	6760602R
• Phosphotyrosine (PT66) Assay Kit	6760620	6760620C	6760620M	6760620R

Selected AlphaScreen References

AlphaScreen General

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Enzyme Assays

- Application Note: P-Tyr-100 Insulin Receptor Tyrosine Kinase Assay **(PerkinElmer Literature Application Note Ref# K4706)**.
- Application Note: Map Kinase Assay **(PerkinElmer Literature Application Note Ref# K4707)**.
- Application Note: Minimizing Reagent Use in the AlphaScreen P-Tyr-100 Assay **(PerkinElmer Literature Application Note Ref# K4708)**.
- Application Note: C-Src Kinase (p60c-src) Assay **(PerkinElmer Literature Application Note Ref# AN003-ASc)**.
- Application Note: Helicase Assay. **(PerkinElmer Literature Application Note Ref# AN006-ASc)**.
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