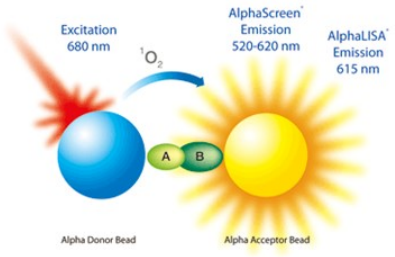
Introduction

AlphaScreen technologies from PerkinElmer are bead-based proximity assays. The binding of molecules captured by donor and acceptor beads leads to an energy transfer via a singlet oxygen species. If the interaction distance is within 200nm, excitation of the donor will make singlet oxygen that will excite the acceptor bead and produce a signal.



The difference between AlphaScreen, AlphaLisa and AlphaPlex is the nature of the acceptor bead. Each uses a streptavidin donor bead, but a different fluorophore, or set of fluorophores, resulting in different emission signals.

Assay	Fluorophore	Emission Wavelength (nm)
AlphaScreen	Thioxene, Anthracene	520-620
AlphaLisa	Europium Chelate	615
AlphaPlex	Samarium/ Terbium	645/545

AlphaPlex is often used in conjunction with AlphaLisa assays. Such multiplex assays can allow for faster analysis times, smaller sample volumes, higher accuracy and reproducibility than single-plex assays.

In general, an Alpha assay works as follows:

- Anti-analyte biotinylated antibodies will bind the streptavidin donor beads.
- Complementary anti-analyte antibodies conjugated to acceptor beads will bring both beads into close proximity, allowing energy transfer and chemiluminescent light emission.

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Synergy Neo2 AlphaScreen, AlphaLisa & AlphaPlex

Amplified Luminescent Proximity Homogeneous Assay (ALPHA)

- Quantify biological interactions (Protein-protein, receptor-ligand etc.) and molecular interaction characterization (k_d).
- High sensitivity: detection at femtogram levels of target.
- Customizable donor and acceptor beads.
- Can detect individual components in large complexes via wide range of fluorophores.

Biotek Synergy Neo2 AlphaScreen Detection Sensitivity Determination

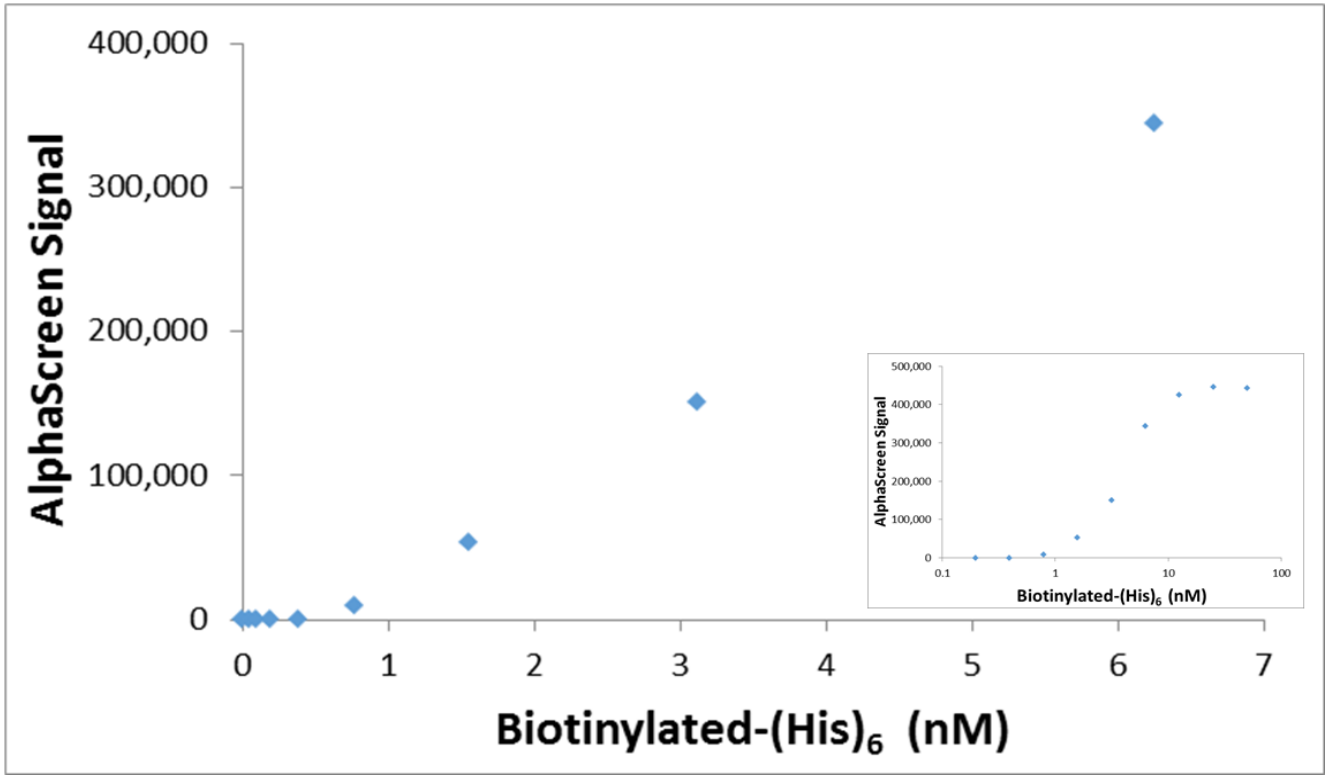
AlphaScreen

Stock Beads: Nickel Chelate Acceptor Beads (PerkinElmer, #AL108C, 20 µg/mL), Streptavidin Donor Beads (PerkinElmer, # 6760002S, 20 µg/mL)

Buffer: 25mM HEPES pH 7.4, 100m NaCl, 0.05% Proclin-300 (Biocide).

Procedure

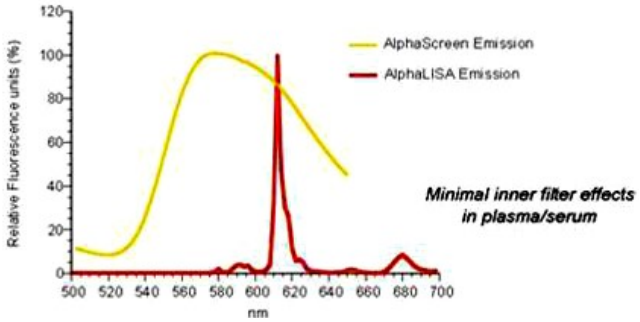
- Dilute stock Nickel Chelate Acceptor Beads with buffer to 50µg/mL, then add 10uL to one row of wells in a 96-well plate.
- Serial dilute stock Biotinylated-$(His)_6$ (See Table 1) and add 5µL to each well containing Nickel Chelate Beads. Incubate covered in the dark for 30 minutes.
- Dilute stock Streptavidin Donor Beads with buffer to 50µg/mL. Add 10µL to each well containing Nickel Chelate Beads/Biotinylated-$(His)_6$ and incubate covered in the dark for a further 60 minutes.
- In duplicate, add 10µL mixture to low-volume, black bottom 384-well assay plate. Centrifuge for 30 seconds at 1000rpm.
- Read fluorescence on Biotek Synergy Neo2 using the AlphaScreen filter, and AlphaScreen preset method.



Additional features of Neo2 AlphaScreen Assay

The AlphaScreen assay uses the NEO laser in the Synergy which is more powerful than a Xe flash lamp and noticeably increases the sensitivity of the assay, allowing for detection of sample in the high pM range.

Additionally, the donor beads have many ways to bind your target including antibody based binding, streptavidin binding, Ni- NTA binding and more, which offers versatility when developing assays. The different donor beads are available commercially through PerkinElmer.



AlphaLisa & AlphaPlex

The whole range of Alpha-based systems use the same technology, but differ in terms of the emitting fluorophore. AlphaLisa uses a fluorophore that emits light in a narrower range and is less susceptible to interference by compounds that absorb light between 500-600 nm. AlphaPlex is a refinement of AlphaLisa that allows for multiplexed detection of biomarkers by using a mix of different fluorophores on donor beads.

Potential applications

- Functional GPCR assays — cAMP quantitation
- Protein-DNA binding assays (EMSA)
- Epigenetics via ChIP-seq related experiments
- Biomarker/cytokine detection
- Antibody binding (ELISA)
- Antibody characterization (Isoytping)
- Targeting of posttranslational modifications of proteins (Acetylation, Glycosidation, methylation and more)
- Binding of target from complex matrices such as cell lysates, serum, plasma, etc.
- Homogenous phage display