

AlphaScreen® Omnibeads

Introduction

The AlphaScreen Omnibeads are designed for the regular calibration and/or normalization of instruments used with the AlphaScreen technology, such as liquid handling systems and multiplate readers. They are also a cost-effective tool for troubleshooting an instrument's malfunctions. Using the AlphaScreen Omnibeads is simple: beads are dispensed and the AlphaScreen signal is read immediately after. The Omnibeads contain all the chemicals necessary for the generation of a strong AlphaScreen signal without the need of an Acceptor bead.

The **Hidex Sense platereader** will help your lab become more effective. The touch screen user interface makes the operation safe and comfortable. Straightforward application focused operation minimizes time spent on instrument training, and is essential for superior results. Detection of the AlphaScreen signal requires a use of two technologies. The donor is excited with Fluorescence and the signal emitted by the acceptor is detected with High-Sensitive Luminescence.

Materials

AlphaScreen Omnibeads (Product number: 6760626D, PerkinElmer) 5 mg/mL
HEPES 1M (Cat. No. BE-17-737E, Lonza)
Tween 20 (P-1379, Sigma)
Diluent 5 mM HEPES + 0,03% Tween 20
White 384 well microplate, round bottom, NBS coating, no lid (Cat. No. 3673, Corning)
680bw40 nm filter (Ferroperm)
Dichroic mirror for AlphaScreen (Hidex)
Hidex Sense Beta (425-311)

Hidex Sense Multitechnology platereader

The Hidex Sense Beta Plus microplate reader uniquely combines liquid scintillation, beta and gamma counting, high sensitivity luminescence and all common non-radioactive detection technologies including spectral photometric detection into one very compact instrument.



Hidex Sense with a touchscreen computer.

Utilizing filter technology for fluorescence applications gives the best possible sensitivity and reading speed. The filter storage provides full flexibility with capacity for 32 filters. Uniquely the same filters can be used for both excitation and emission with unlimited flexibility.



AlphaScreen Assay principle

AlphaScreen® is bead-based assay technology used to study biomolecular interactions in a microplate format. The acronym "Alpha" stands for amplified luminescent proximity homogeneous assay. As the name implies, some of the key features of these technologies are that they are non-radioactive, homogeneous proximity assays. Binding of molecules captured on the beads leads to an energy transfer from one bead to the other. ultimately producing a luminescent signal.

Donor beads contain a photosensitizer, phthalocyanine, which converts ambient oxygen to an excited and reactive form of O₂, singlet oxygen, upon illumination at 680 nm. Please note that singlet oxygen is not a radical; it is molecular oxygen with a single excited electron. Like other excited molecules, singlet oxygen has a limited lifetime prior to falling back to ground state. Within its 4 µsec half-life, singlet oxygen can diffuse approximately 200 nm in solution. If an Acceptor bead is within that proximity, energy is transferred from the singlet oxygen to thioxene derivatives within the Acceptor bead, subsequently culminating in light production at 520-620 nm. In the absence of an Acceptor bead, singlet oxygen falls to ground state and no signal is produced. This proximity-dependent chemical energy transfer is the basis for AlphaScreen's

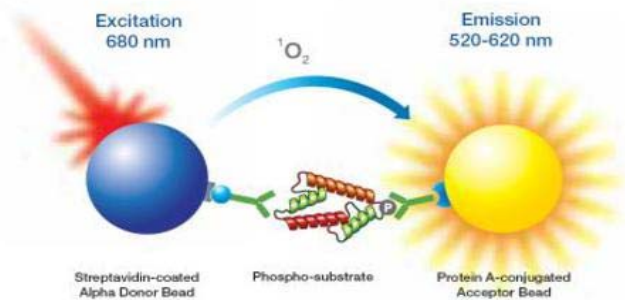


Fig. 1. AlphaScreen assay principle.

Reagents

Diluent 5 mM HEPES + 0,03% Tween 20

Omnibead dilutions

Dilute the stock solution 50-fold with the diluent

Prepare a 2-fold dilution series

0,78 µg/mL

1,56

3,1

6,3

12,5

25

50

100

Reader setup

Dual reporter assay: 1 well per batch

Fluorescence

Excitation filter 682/40 nm

Lamp power Medium

Flashes 500

Ex. aperture 8

High-sensitivity Luminescence

CTime 1 s

Focus 8,5

Test procedure

Dispense Diluent and Omnibeads dilutions 25 µL/well in duplicates.

Select the wells to be measured in the AlphaScreen assay template and press "Run now".

If the template is not included in the PlateReader software, it is available from Hidex.

Note!

Omnibeads are very light-sensitive and even if exposed to normal room lightning, emit high luminescence signal. Therefore, to eliminate high background counts, plate should be kept in dark (e.g. in a platereader) for at least 10 minutes prior to starting of a measurement.

Results

Average CPS values of the duplicates were calculated and after background subtraction, these values were plotted against Omnibeads concentration (Fig. 2A.). Typically the bead concentration is 20 µg/mL in AlphaScreen assays. Therefore, the lower range of the dose-response relationship is shown separately (Fig. 2B).

Conc (µg/mL)	CPS1	CPS2	X	X-Bkg
0			83	0
0,78	4004	4040	4022	3939
1,6	7521	7975	7748	7665
3,1	14171	15661	14916	14833
6,3	32855	30381	31618	31535
12,5	59734	61667	60701	60618
25	110691	118362	114527	114444
50	213242	229113	221178	221095
100	413828	427393	420611	420528

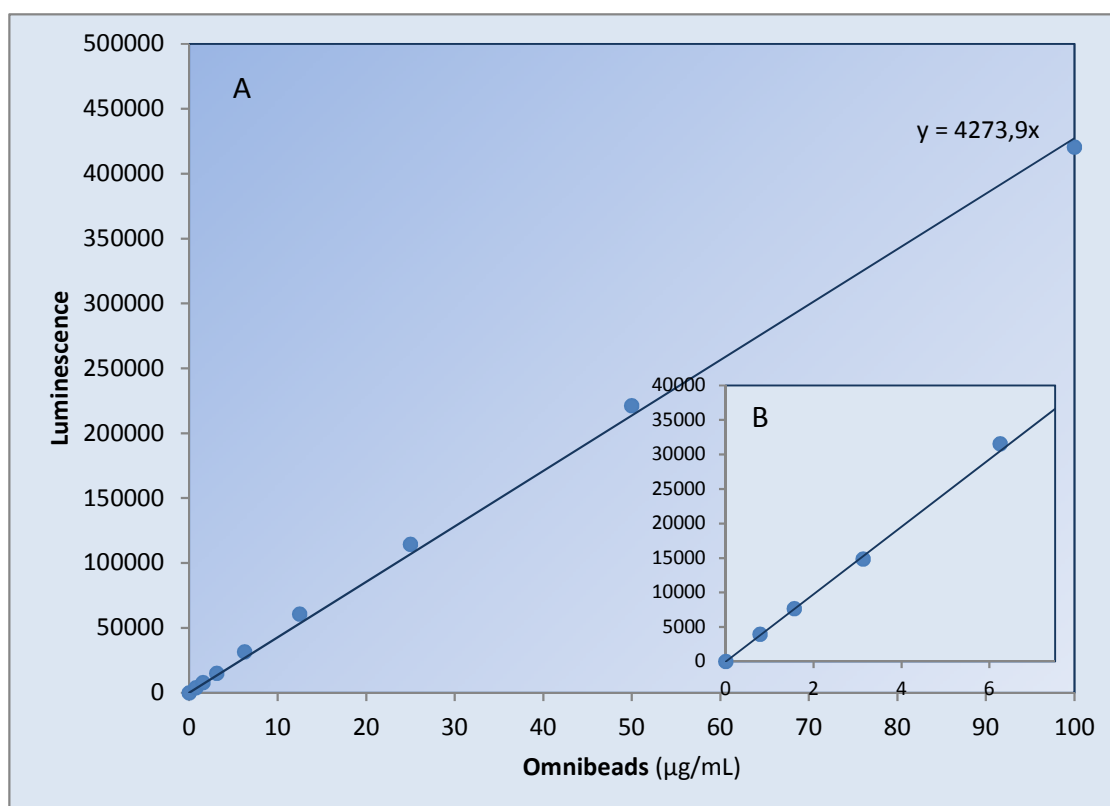


Fig. 2. Omnibead linear dose-response relationship (A), low range (B).

Detection limit

The calculation of the detection limit (DL) is based on the 3*standard deviation (3SD) of the background signal and the slope (S) of the dilution line: $DL = 3SD/S$.

Background	Avg.	83	CPS
	3SD	34	CPS
Det. Limit (µg/mL)		0,008	µg/mL

Conclusions

Hidex Sense Beta combines fluorescence excitation and high-sensitivity luminescence detection required in AlphaScreen assays resulting in a very low detection limit. The reader together with easy-to-use touch screen userinterface makes the operation safe and comfortable. Straightforward application focused operation minimizes time spent on instrument training, and is essential for superior results.

Hidex Sense AlphaScreen package including a filter, dichroic mirror and assay protocol is available on request.

AlphaScreen® is a trademark of PerkinElmer