PerkinElmer®

AlphaLISA immunoassays: the no-wash alternative to ELISAs for research and drug discovery

PerkinElmer's bead-based AlphaLISA® immunoassays are designed for the detection of analytes in biological samples. These chemiluminescent, no-wash assays are ideally suited for miniaturization and automation. They exhibit remarkable sensitivity, wide dynamic range and robust performance that compares advantageously with conventional enzyme-linked immunosorbent assay (ELISA).

ELISA is the most widely used detection platform for the quantification of analytes in biological samples. Because they require multiple washes, ELISAs are difficult to adapt to high throughput and automation. Their relatively narrow dynamic range often requires testing more than one sample dilution. There is clearly a need for simple and more robust alternatives for the quantification of biomarkers in a high-throughput screening format. The new AlphaLISA platform has been specifically designed for that purpose for both the research and drug-discovery fields.

The AlphaLISA bead-based technology relies on PerkinElmer's exclusive amplified luminescent proximity homogeneous assay (AlphaScreen®) and uses a luminescent oxygen-channeling chemistry[1]. AlphaLISA protocols can be set up as sandwich or competition immunoassays. In a sandwich assay (**Fig. 1**), an analyte is captured by a biotinylated antibody bound to streptavidin-coated donor beads and a second antibody conjugated to AlphaLISA acceptor beads. The binding of the two antibodies to the analyte brings donor and acceptor beads into proximity. Laser irradiation of donor beads at 680 nm generates a flow of singlet oxygen, triggering a cascade of chemical events in nearby acceptor beads, which results in a chemiluminescent emission at 615 nm. In competitive AlphaLISA immunoassays, a biotinylated analyte bound to streptavidin donor beads is used with an antibody conjugated to AlphaLISA acceptor beads.

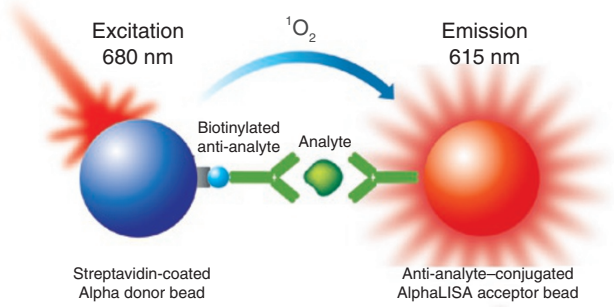

Figure 1 | Principle of the AlphaLISA technology. A biotinylated antibody to the analyte binds to the streptavidin-coated donor beads and a second antibody to the analyte is directly conjugated to AlphaLISA acceptor beads. In the presence of the analyte, the two beads come into close proximity. The excitation of the donor beads at 680 nm generates singlet oxygen molecules that trigger a series of chemical reactions in the acceptor beads resulting in a sharp peak of light emission at 615 nm.

Rapid and simple quantification of analytes

AlphaLISA assays are performed following simple 'mix-and-measure' protocols with reduced hands-on and total assay times compared to ELISAs (**Fig. 2**). Homogeneous AlphaLISA assays eliminate the need for multiple washes to separate bound from unbound assay components.

Miniaturization and automation

Miniaturization is a key consideration for reducing screening cost and increasing throughput during the drug-discovery process. AlphaLISA assays are truly miniaturizable and automatable, with

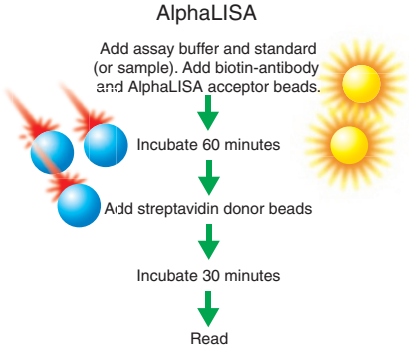

Figure 2 | AlphaLISA assay protocol. AlphaLISA assays are conducted using a simple mix-and-read protocol that substantially reduces assay development and hands-on time, while improving throughput and ease of automation.

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no loss in sensitivity. This feature is crucial when quantitative detection of analytes must be performed on precious samples available in a limited quantity. AlphaLISA assays are highly robust when using sample volumes as low as 1 µl in total assay volumes of 10 µl. Assays can be performed routinely in 96- or 384-well microplates or scaled down to 1,536-well-plate format. Fully validated AlphaLISA protocols are available for PerkinElmer's JANUS® automated workstation to automate assays in high-throughput screening format.

Versatility of AlphaLISA assays

AlphaLISA assays are ideal for measuring analytes in a wide variety of samples, including cell culture supernatants, crude cell lysates, serum and plasma[2,3]. They allow quantification of proteins that are secreted, intracellular or membrane-bound. Assays illustrating this versatility are presented in **Figure 3**. These four sandwich-type assays use a biotinylated antibody and a second antibody directly coupled to the acceptor beads.

We obtained AlphaLISA standard curves using AlphaLISA kits for human amyloid β1-40 and β1-42 peptides (**Fig. 3a**). The lower detection limit (LDL) for the human amyloid β1-40 and β1-42 peptides was 50 and 110 pg ml^{-1}, respectively, with a wide dynamic range of 3 log units. We also obtained a standard curve using an AlphaLISA OnPoint™ custom assay developed for the quantification of a recombinant therapeutic antibody (**Fig. 3b**). This custom assay achieved a high sensitivity (LDL of 0.5 ng ml^{-1}). Quantification of therapeutic antibodies is essential both in the drug development phase and later in clinical studies.

AlphaLISA assays can also be used for detecting post-translational modifications of intracellular targets. We performed a *Z*'-value analysis[4] for an assay measuring the dephosphorylation of an intracellular target in lysates from unstimulated cells and those treated with an anticancer drug (**Fig. 3c**). In this custom assay setup, we used a biotinylated antibody to phosphorylated target, and a second antibody coupled directly to the acceptor beads and recognizing all forms of the target. This assay had a *Z*′-value of 0.9, demonstrating remarkable assay robustness.

The AlphaLISA technology is also suitable for detecting integral membrane proteins. We detected expression of the epidermal growth factor receptor (EGFR) in A431 cells, a cell line derived from a human epidermoid carcinoma overexpressing EGFR (**Fig. 3d**). We also evaluated expression of EGFR in human embryonic kidney (HEK-293) cells. In this assay, we washed the cells with PBS to remove secreted EGFR extracellular domain in the culture medium and then added the AlphaLISA reagents directly into the culture wells for a simple, all-in-one-well assay. We obtained LDLs of 42 and 1,624 cells for the A431 and HEK-293 cell lines, demonstrating assay sensitivity. The relative expression of EGFR in the two cell lines was in agreement with published data[5].

Our results demonstrate that AlphaLISA assays are highly selective and sensitive. This selectivity is attributable to the careful selection of pairs of analyte-specific antibodies. AlphaLISA protocols require small sample volumes, on the order of 1–5 µl, and yet their detection limit is equivalent to or lower than that of ELISAs using sample volumes of up to 200 µl. This high sensitivity is mostly due to the nature of the AlphaLISA detection platform: the flow of singlet oxygen produced upon donor beads irradiation induces remarkable signal amplification in nearby acceptor beads. Moreover, the high antibody density on beads creates an avidity phenomenon, increasing sensitivity. Indeed, in a direct comparison of AlphaLISA assays and ELISAs for measuring human insulin in plasma samples, the AlphaLISA assay detected 15-fold less analyte than ELISA, while using one-fifth the sample volume[6].

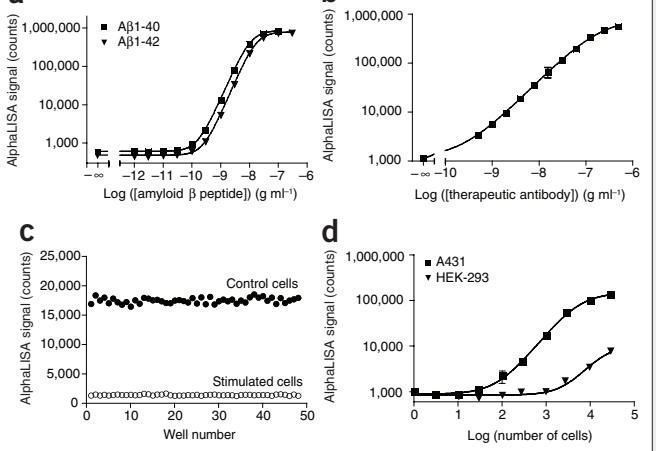

Figure 3 | Detection of a variety of analytes using the AlphaLISA assay technology. (**a**–**d**) The AlphaLISA assay platform was used to evaluate the lower detection limit for amyloid β1-40 (Aβ1-40) and amyloid β1-42 (Aβ1-42) peptides (**a**), detect a recombinantly expressed therapeutic antibody (**b**), evaluate the dephosphorylation of an intracellular target after cell stimulation with an anticancer drug (**c**) and determine expression of the EGFR in A431 epidermoid carcinoma and HEK-293 cells in an all-in-one-well assay (**d**).

Conclusions

AlphaLISA assays are homogeneous, no-wash immunoassays with high sensitivity and wide dynamic ranges. Compared to standard ELISA protocols, AlphaLISA assays increase throughput while substantially decreasing hands-on and total assay times. AlphaLISA assays are versatile and can be used to detect analytes that are secreted, intracellular or membrane-bound. In addition to providing high-quality data and robust performance, AlphaLISA assays are simple and quick to optimize. They are miniaturizable and automatable for increased laboratory productivity. The AlphaLISA platform is thus an ideal for the immunodetection of biomarkers and is now emerging as the new-generation immunoassay technology in drug discovery, preclinical studies and basic research.

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