Development of Customized Immunoassays using AlphaLISA™

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1 Abstract

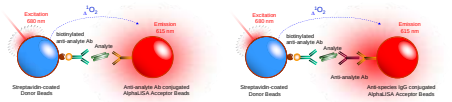
Competitive and non-competitive (sandwich) immunoassays, such as ELISA assays, are widely employed in laboratories to measure the concentration of selected proteins in biofluids, typically serum, plasma, urine, cell lysates and cell culture supernatants. These assays take advantage of the specific binding of an antibody to its antigen. The recently introduced AlphaLISA technology provides the advantage of specificity of an immunoassay without the disadvantages associated with wash assays (tedious assays with long incubation times and low throughput densities). The high versatility and sensitivity features make the AlphaLISA platform an excellent choice for the rapid conversion of any ELISA tests to cost-effective miniaturized homogeneous assays.

This poster presents key examples of AlphaLISA assays that were developed for the detection of various analytes: from the measurement of biomarkers in serum/plasma samples, to the detection of phosphorylated targets in cell lysates, analysis of secreted proteins from cell culture supernatants, as well as the detection/dosing of therapeutic antibodies. AlphaLISA has been demonstrated to be a remarkable analytical method combining multiple benefits and allowing for quick and easy implementation, especially in high throughput screening laboratories. PerkinElmer offers its expertise in assay development to scientists for the conversion of any immunoassays to AlphaLISA assays.

2 AlphaLISA Sandwich Immunoassays

A - Direct Capture Configuration

B - Indirect Capture Configuration



AlphaLISA sandwich assays can be designed in a direct or indirect antibody capture configuration.

Panel A: Direct capture configuration: A biotinylated anti-analyte antibody binds to the Streptavidin-coated Donor Beads while another anti-analyte antibody is directly conjugated to AlphaLISA Acceptor Beads. In the presence of the analyte, the two types of beads come into close proximity. The excitation of the Donor Beads at 680 nm provokes the release of singlet oxygen molecules that trigger a cascade of energy transfer to the Acceptor Beads, resulting in a sharp peak of light emission at 615 nm. The orientation of the antibodies on the two beads can be reversed.

Panel B: Indirect capture configuration: In this case, one of the anti-analyte antibodies is captured by a secondary anti-species antibody. Different antibody orientations are also possible with this set-up.

3 AlphaLISA *versus* ELISA Assays

AlphaLISA

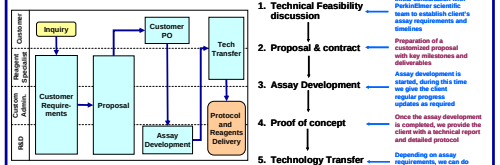
- No wash steps
- Fewer incubations
- Shorter total assay time
- Higher throughput
- Reduced assay costs

4 AlphaLISA Applications

- Detection and quantification of multiple targets in various samples:
  - Biomarkers (serum or plasma)
  - Phosphorylated intracellular proteins (cell lysates)
  - Intracellular proteins (cell lysates)
  - Secreted proteins (cell culture supernatants)
  - Purified proteins
  - Immunotherapeutics
- Purity assessment of therapeutic antibody preparations (Host Cell Proteins and residual Protein A assays)
- New AlphaLISA products available soon from PerkinElmer, Inc.:
  - Stand-alone conjugated AlphaLISA beads (toolbox products)
  - Immunoassay kits for detection of various analytes

5 Custom Assay Development Process

On>Point Services — *Phase-Gated Process*



6 Materials and Methods

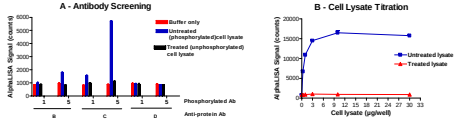
Materials – Specific anti-analyte antibodies and analyte are either purchased from antibody providers or supplied by the customer. Samples (cell lysates, serum samples etc.) are provided by the customer. AlphaLISA Acceptor Beads, Streptavidin-coated Donor Beads and AlphaLISA Assay Buffer, and AlphaLISA lysis buffers are provided by PerkinElmer.

Antibody Coupling to AlphaLISA Acceptor Beads – Antibodies are conjugated on the AlphaLISA Acceptor beads via reductive amination with the aldehyde reactive groups on the beads and the primary amines on the antibody. The procedure is fast and simple.

Antibody Biotinylation – Antibodies are biotinylated with a biotinylating agent using standard procedures.

General Assay Procedure - AlphaLISA assays are performed at room temperature in White OptiPlate™-96 or -384 microplates (PerkinElmer) in final volumes of 25-50 µL. A typical assay procedure is presented in Panel 3, however the assay conditions are optimized for each assay. The microplates are read using an EnVision® Multilabel Reader equipped with an excitation filter of 680 nm and an emission filter of 615 nm (PerkinElmer).

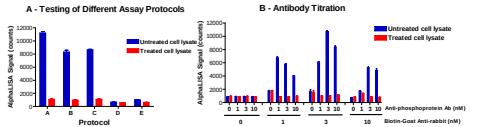
7 Detection of a Phosphorylated Cellular Target - Feasibility Phase



This phosphorylated intracellular tyrosine protein was detected in an indirect AlphaLISA configuration using biotinylated goat anti-rabbit captured by streptavidin-Donor beads, rabbit anti-phosphoprotein antibody, anti-protein antibody and AlphaLISA Acceptor beads. During the Feasibility Phase, the anti-protein and anti-phosphoprotein antibodies were screened, i.e. all possible antibody combinations were tested in AlphaLISA assays with untreated (phosphorylated) and treated (non phosphorylated) human stomach cancer cell lysates (see Panel A), with the goal to identify the optimal antibody pair giving a robust signal and meeting the assay requirements defined by the customer. In this particular case, the selected antibody pair was composed of biotin-Ab #5 and anti-protein antibody #C.

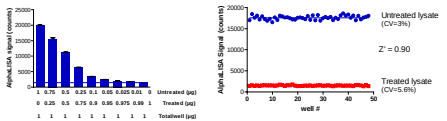
In Panel B, the cell lysate was titrated; 1 µg/well, producing a S/B of 17, was used in subsequent experiments.

8 Detection of a Phosphorylated Cellular Target - Optimization Phase



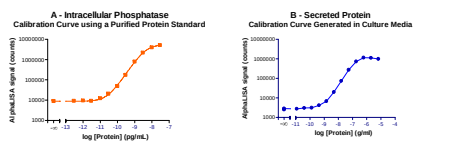
Typically, several assay parameters are tested in the Optimization Phase, including the order of reagent addition, reagent concentration, assay volume and incubation times. The goal of this Phase is to develop the simplest and most performant assay for measuring the analyte of choice. In this figure, we present only two of the several assay optimization datasets obtained with this phosphorylated kinase. In Panel A, five assay protocols using different orders of reagent addition were tested: Protocol A was shown to be the most performant, while protocols D and E where the biotinylated antibody is premixed with the streptavidin-Donor beads, did not produce any specific response. In Panel B, the anti-phosphokinase and biotinylated goat anti-rabbit antibody were titrated. The optimal concentrations observed were 3 nM for both antibodies.

9 Detection of a Phosphorylated Cellular Target – Final Assay Characteristics



After optimization, the assay characteristics were determined using the final assay conditions. The detection limit of the assay (LDL) was determined by titration of untreated cell lysate while keeping constant the total amount of cell lysate per well at 1 µg using treated cell lysate (see Panel A). The LDL was determined to be 0.01 µg/well of untreated lysate. Finally, to assess the robustness and reproducibility of the assay, a Z' determination study was performed on two populations of data generated with the untreated and treated cell lysates. The Z' value of 0.9 obtained demonstrates that the AlphaLISA assay developed for this phosphorylated intracellular protein is suitable for HTS applications.

10 Detection of Intracellular and Secreted Protein Targets

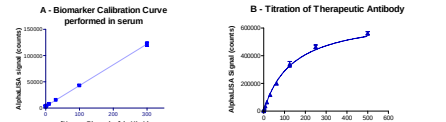


The two AlphaLISA detection assays presented here are direct sandwich assays consisting of the capture of the target proteins by two antibodies directed against diverse epitopes.

Panel A: Calibration curve for an intracellular phosphatase.

Panel B: Calibration curve for a protein secreted in the culture medium: to mimic the sample matrix, the curve was generated in the culture medium.

11 Quantification of Biomarker Levels in Serum & Immunotherapeutics Detection



Panel A: The biomarker of interest was diluted in human serum and titrated in an AlphaLISA direct sandwich assay. The performance parameters of the assay were determined (ex. LDL = 1.3 mU/mL) and found to be comparable to published ELISA assay parameters.

Panel B: In this case, the analyte is a therapeutic antibody that was titrated in an AlphaLISA direct sandwich assay. High sensitivity (0.5 ng/mL) and wide dynamic range (4 log units) were achieved in a few optimization experiments.

12 Conclusion

- Typical AlphaLISA data generated during Custom Assay Development Projects are presented to demonstrate the superior performance of the AlphaLISA platform when developing immunoassays.
- It is now well established that AlphaLISA is a non-wash ELISA replacement platform perfectly suited for the detection and quantification of a wide variety of analytes based on sandwich or competitive immunoassays.
- Our Custom Assay Development Service offers fast, easy and affordable high-quality service for the conversion of immunoassays to the AlphaLISA platform. Our dedicated team of expert scientists are strongly focused to achieve the goal of fulfilling customer assay requirements.
- **Over 30 assays have already been developed for more than 10 biopharmaceutical clients.**

References:

- AlphaLISA Assay Development Guide, PerkinElmer Inc.
- Insulin Detection Assay using AlphaLISA Acceptor Beads, Application Note, PerkinElmer Inc.
- A Luminescent Oxygen Channeling Immunoassay for the Determination of insulin in Human Plasma. Poulsen, F. and Jensen, B. J. of Biomolecular Screening, January 2007.