AlphaLISA Assays are Homogeneous Sensitive Immunoassays for Detection of Analytes in a Variety of Biological Matrices

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Abstract
The AlphaLISA® assay is a homogeneous immunoassay alternative to classical ELISA. AlphaLISA assays were originally utilized to detect analyte in cell culture supernatants or serum/plasma samples. More recently, AlphaLISA technology has been applied to detect analytes in biological samples or fluids, which include extracts from cultured cells, and fluids and tissue homogenates from animals. We report the development of an assay to measure active caspase-3 in cell lysates from both suspension and adherent cell models (Jurkat and HeLa, respectively). For compound screening, cells are treated with test material and subsequently lysate buffer is added. After a short incubation, target-specific AlphaLISA reagents are directly added, providing a highly efficient all-in-one well assay format. Under optimal conditions, signal to background (S/B) values up to 17 and 2.0 values up to 0.8 were obtained with zymotopin-streutated Jurkat cells. Assays were also developed for biological samples derived from rodents or humans. Quantification of analytes in animal tissue extracts or biological fluids requires an appropriate diluent so that samples can be accurately extracted. We also report the optimization of the assay, recovery of spiked analytes was generally in the range of 70 to 130%. As examples, mouse interleukin 6 was measured in bronchoalveolar lavage fluid (BALF) at a level of 20 pg/mL, human amyloid beta 1-42 peptide was measured in cerebrospinal fluid (CSF) at a level of 0.3 ng/mL, and mouse vascular endothelial growth factor (VEGF) was detected in lung homogenates at a level of 1 ng/mL. In general, a major advantage of using AlphaLISA for the development of analytic samples is that sample volumes as large as 2.5 µL can be utilized. Due to the absence of wash-steps, greatly reduces the assay time and improves the reproducibility of the data.

1 Materials and Methods
Analyte detection
AlphaLISA kits and buffers are all available from PerkinElmer. All calibration curves were done using analyte and reagents supplied in the kits, and using optimal protocols available in the Technical Data Sheets.

2 Expanding Applications
Biological samples or fluids
SI-based biological samples or fluids were supplied as pools of individuals (for assay development), or separate samples from 10 to 20 individuals, non-medicated, non-treated, from Biorange LLC:
- Mouse bronchial lavage fluid (BALF): Strain Sprague Dawley, cat# RAT-BROLAV
- Beagle bronchial lavage fluid (BALF): cat# BGL-BROLAV
- Rat bronchial lavage fluid (BALF): Sprague Dawley, cat# RAT-BROLAV
- Human bronchial lavage fluid (BALF): Sprague Dawley, cat# HUM-BROLAV
- Lung homogenates: Strain Sprague Dawley, cat# MUS-LIH-TOUC

All biological samples or fluids were supplied as pools of individuals (for assay development), and shown to detect endogenous level of cytokine. Analytes were optimized by choosing the correct matrix for the calibration curve values and inter-assay precision can be achieved. Quantitative assays for detecting mouse TNF and human VEGF were optimized by choosing the correct matrix for the calibration curve and shown to detect endogenous level of cytokine. VEGF and TNF were robustly detected in BALF and homogenates. Excellent recovery for all 3 spikes tested in 1/2 mouse lung homogenates. Measured VEGF in other biological matrices from different species. Different types of analytes such as small physiological level in mouse tissue.

3 Alpha Technology Assay Principle
AlphaLISA Assays are Homogeneous Sensitive Immunoassays for Detection of Analytes in a Variety of Biological Matrices

4 Expanding Applications

5 Active Caspase-3 Cellular Assay

6 Active Caspase-3 Assay Precision

7 Mouse TNFα in BALF

8 Mouse VEGFα in Lung Homogenates

9 Conclusion
AlphaLISA technology offers the possibility to accurately detect physiologic levels of analytes even if very low volume of fluid is collected per animal.