New AlphaLISA assays for the development of monoclonal antibody biotherapeutics

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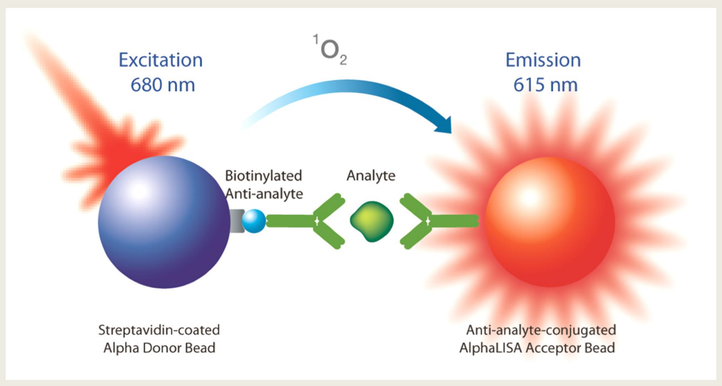
1 Abstract

There is an increasing need for efficient assays to detect and characterize therapeutic monoclonal antibodies during various stages of development. AlphaLISA® technology offers a streamlined work flow for performing immunoassays in a more productive and cost efficient manner. To add to the breadth of available AlphaLISA assays for these applications, we established new reagents to quantitate: monoclonal antibodies of the IgG1 or IgG4 subclasses, CHO and PER.C6® host cell protein contaminants, and core α1,6-fucosylated IgG.

2 Introduction

Immunoassays are commonly used in the development and characterization of monoclonal antibody biotherapeutics (TAbs). There is a current need for simplified and sensitive assays that improve the workflow in biotherapeutics labs. The most common technology for performing immunoassays is enzyme-linked immunosorbent assays (ELISA), which is a robust method but requires multiple steps, is time consuming and can lead to variable data. The AlphaLISA format is an alternative to ELISA; it is a chemiluminescent homogeneous bead-based technology, which therefore does not require washing or separation steps. The assays are typically performed in 96- or 384-well plates. When only small amounts of sample are available, sample volumes as low as 5 µL can be utilized. The total assay time is less than 3 hours, which is half the time of a normal ELISA. A generic assay has been developed to quantitate therapeutic antibodies using anti-human IgG1 or IgG4 antibodies to sandwich the target. Other immunoassays have been developed to detect bioprocess contaminants such as host cell proteins (CHO and PER.C6® HCP). An assay to specifically detect fucosylated IgG has also been developed by use of an anti-IgG antibody and a core fucose-specific lectin. Overall, the AlphaLISA technology provides a versatile assay platform for replacing various assays in the biotherapeutics labs.

3 AlphaLISA Technology Principle



The biotinylated anti-analyte antibody binds to the Streptavidin-coated Alpha Donor beads while another anti-analyte antibody is conjugated to AlphaLISA Acceptor beads. In the presence of the analyte, the beads come into close proximity. The 680 nm laser excitation of the Donor beads provokes the release of singlet oxygen molecules that triggers a cascade of energy transfer in the Acceptor beads resulting in a sharp peak of light emission at 615 nm.

4 Materials

Immunoassay kits:

The following AlphaLISA kits are available from PerkinElmer: Chinese Hamster Ovary Cell Host Cell Proteins (CHO HCP) (broad reactivity) (AL301), PER.C6® Cell Host Cell Proteins (PER.C6® HCP) (AL302), Human Immunoglobulin G subclass 1 (IgG1) (AL303), Human Immunoglobulin G subclass 4 (IgG4) (AL304). The following Acceptor beads are sold as standalone products: *Lens culinaris* agglutinin (LCA) (AL140), Anti-Human IgG1 (AL141), Anti-Human IgG4 (AL142). The CHO HCP kit (AL210) is currently available.

5 AlphaLISA: quick, simple, precise

5 µL Standard or Sample

↓

Add 10 µL of AlphaLISA Anti-Analyte Acceptor beads

↓ Incubate 30 minutes

Add 10 µL of Biotinylated Anti-Analyte Antibody

↓ Incubate 60 minutes

Add 25 µL of Streptavidin Alpha Donor beads

↓ Incubate 30 minutes

Read at 615 nm using EnVision® or EnSpire® Multimode Plate Reader and analyze data

Typical assay precision data

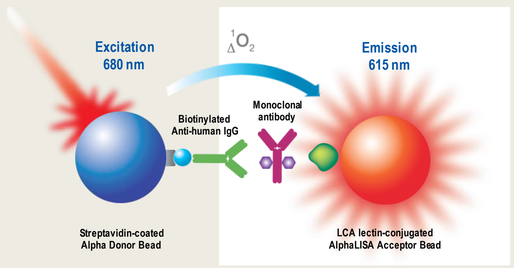
Intra-assay precision			
Sample	Mean (pg/mL)	SD (pg/mL)	%CV (n=18)
A	92 819	6 955	7.5
B	10 765	641	6.0
C	1 049	92	8.8

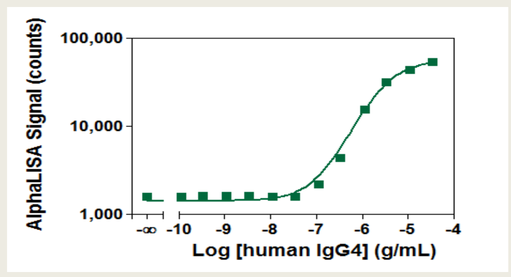
Inter-assay precision			
Sample	Mean (pg/mL)	SD (pg/mL)	%CV (n=6)
A	92 819	10 650	11.5
B	10 765	1 137	10.6
C	1 049	180	17.2

Precision of CHO HCP assay (Cat. no. AL210). Assay precision data were calculated from a total of 18 assays. Two operators performed three independent assays using three different kit lots. Each assay consisted of one standard curve and three control samples of high (A), medium (B) and low (C) concentrations, assayed in triplicate.

6 Fucosylated IgG detection

The carbohydrates or glycans linked on the biotherapeutic antibody may play a major role in the mechanism of action as well as biodistribution of the drug. Detection of core fucose on monoclonal antibodies using Alpha technology can be used for antibody screening and characterization. The fucosylated IgG assay is based on the detection of core fucose by *lens culinaris* agglutinin, which has a high specificity for α1,6-linked fucose [1].





Detection of fucosylated IgG4 in serum-free cell culture medium. Human IgG4 from myeloma plasma was diluted in cell culture medium (Hybridoma-SFM Gibco #12045-084). The fucosylated IgG4 was then detected using a typical AlphaLISA protocol.

7 CHO and PER.C6® HCP assays

The CHO and PER.C6® HCP assays measure the presence of contaminating proteins in purification samples.
Two CHO HCP kits have been developed (AL210 and AL301). The second product is identified as "broad reactivity", because the antibodies have been shown to detect more CHO proteins than the previous kit. Ideally, both assays should be tested for a given process to determine the most suitable. The lower detection limit and upper limit of detection are provided in the following table.

Catalogue number	Kit name	Lower detection limit * (ng/mL)	Upper limit ** (µg/mL)
AL210	CHO HCP	0.2	0.3
AL301	CHO HCP (broad reactivity)	0.5	1
AL302	PER.C6® HCP	0.8	1

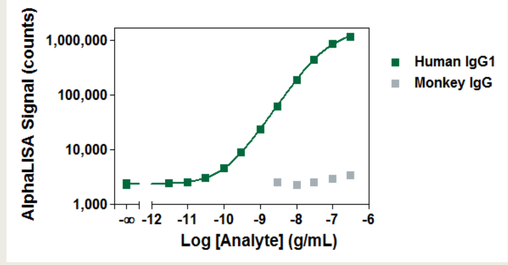
* Calculated by interpolating the average background counts (12 wells without analyte) + 3 x standard deviation value on the standard curve.
** Corresponds to analyte concentration on the standard curve giving maximum signal.

8 Pharmacokinetics of TAbs

During the exploratory and pre-clinical phases, pharmacokinetic studies of TAbs are commonly performed in rodents or monkeys (non-human primates). TAb is injected into the animal and then measured in the serum at different time points. The TAb is measured using a generic IgG assay or a ligand binding assay.

Generic IgG assay

A combination of an anti-human IgG antibody, monkey-adsorbed, and a subclass specific anti-IgG1 or 4 antibody are used to obtain highly sensitive assays to measure IgG1 or IgG4 in animal serum.



Sensitivity and specificity of the human IgG1 assay. Human IgG1 purified from myeloma plasma or total IgG from cynomolgus monkey were diluted in buffer and detected using a typical AlphaLISA protocol. The human IgG1 is detected down to a level of 20 pg/mL, whereas 300 ng/mL of monkey IgG gives only background signal.

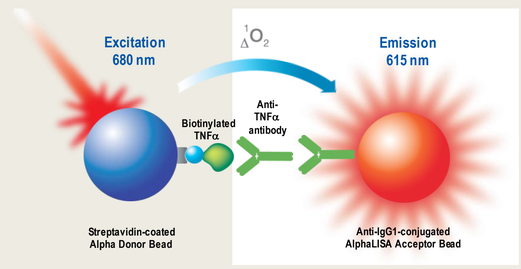
Linearity	
Dilution factor	% Recovery
1	100
2	89
4	83
8	83
16	84

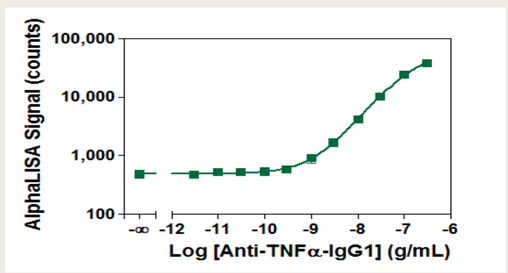
Spike recovery	
Spike (ng/mL)	% Recovery
100	85
10	82
1	86

Accuracy of the human IgG1 assay in monkey serum. 10% monkey serum was used as analyte diluent. A pool of monkey sera spiked with 1000 ng/mL of human IgG1 was initially diluted 10-fold with buffer. This sample containing 100 ng/mL IgG1 was used for the linearity experiment. The recovery for linearity was calculated using the undiluted sample (1X) as the 100% value. For spike recovery, the recovery was calculated based on the theoretical amount of IgG1 present.

Ligand binding assay

Monoclonal antibodies specific to either IgG1 or IgG4 subclass were conjugated to AlphaLISA Acceptor beads to be used for AlphaLISA assays to measure TAb in serum via a ligand binding assay. A human monoclonal antibody specific to TNFα was used as a model to demonstrate the application of the assay for detection of a TAb in monkey serum.





Detection of anti-TNFα antibody in monkey serum. A monoclonal antibody (IgG1 subclass) against TNFα was diluted in 10% monkey serum and detected using anti-IgG1-conjugated Acceptor beads with biotinylated TNFα as ligand. The lower detection limit was 0.3 ng/mL.

9 Summary

Sensitive and reproducible immunoassays were developed for analyses of biotherapeutics. These assays can be applied in different stages of TAb development such as cloning and expression, process development and pharmacokinetic studies.

References
1. Tateno, H. *et al*. Comparative analysis of core-fucose-binding lectins from *Lens culinaris* and *Pisum sativum* using frontal affinity chromatography. Glycobiology 19, 527–536 (2009).

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