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 workflow 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 antibodies (MBAs). There is a 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 transfers energy to the donor in the Acceptor beads resulting in a sharp peak of light emission at 615 nm.

4 Materials

Immunoassays 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 AlphaLISA kits are available for specific binding:
- 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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Intra-assay precision</th>
<th>Inter-assay precision</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>(Mean ± SD) (ng/mL)</td>
<td>(Mean ± SD) (ng/mL)</td>
</tr>
<tr>
<td>a</td>
<td>92 819 ± 6 955</td>
<td>7.8</td>
</tr>
<tr>
<td>b</td>
<td>10 765 ± 441</td>
<td>6.0</td>
</tr>
<tr>
<td>c</td>
<td>1 049 ± 92</td>
<td>8.8</td>
</tr>
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</table>

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 AlphaLISA 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.

Detection of fucosylated IgG4 in serum-free cell culture medium. Human IgG4 from myeloma plasma was diluted in cell culture medium (Hybridoma-SFM Glico [12049-084]). 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.

<table>
<thead>
<tr>
<th>Catalogue number</th>
<th>Kit name</th>
<th>Lower detection limit (ng/mL)</th>
<th>Upper limit (μg/mL)</th>
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<tbody>
<tr>
<td>AL210</td>
<td>CHO HCP</td>
<td>0.2</td>
<td>1</td>
</tr>
<tr>
<td>AL301</td>
<td>CHO HCP (broad reactive)</td>
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<td>1</td>
</tr>
<tr>
<td>AL302</td>
<td>PER.C6® HCP</td>
<td>0.8</td>
<td>1</td>
</tr>
</tbody>
</table>

8 Pharmacokinetics of TAbs

During the exploratory and pre-clinical phases, pharmacokinetic studies of TAbs are usually 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 specific 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 monkeys were diluted in buffer and detected using a typical AlphaLISA protocol. The human IgG1 is detected down to a level of 20 ng/mL, whereas 380 ng/mL of monkey IgG gives only background signal.

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.

9 Summary

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

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