Use of the automated Janus® workstation to develop efficient AlphaLISA immunoassays for isotyping human monoclonal antibodies.

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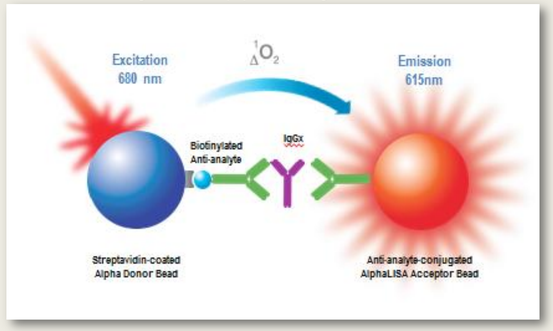
1 Introduction

Immunoassays are commonly used in determining the isotype of monoclonal antibody produced via hybridoma technology. There is an increasing need for efficient and sensitive assays to determine the isotype of these monoclonal antibodies. The most common technology for performing immunoassays is the enzyme-linked immunosorbent assay (ELISA), which is a time-proven method but requires multiple and time-consuming steps that can lead to variable data. The AlphaLISA® assay format is a chemiluminescent homogeneous bead-based technology which does not require washing or separation steps, and as such provides significant advantages over ELISA. AlphaLISA assays are typically performed in 96- or 384-well plates and can be run with sample volumes as low as 5 µL, conserving valuable test materials. The total assay time is less than 3 hours, which is half the time of the most common ELISA. Herein, we report the use of automation to develop several new AlphaLISA assays to isotype human IgG monoclonal antibodies produced in hybridomas.

2 AlphaLISA Technology Principle and Integration of Laboratory Automation

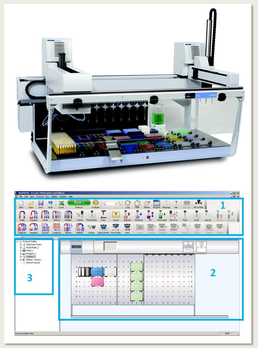
The biotinylated anti-analyte antibody binds to the Streptavidin-coated Alpha Donor beads while another anti-analyte antibody is conjugated to AlphaLISA Acceptor beads. In the presence of the analyte, the beads come into close proximity. The 680 nm laser excitation of the Donor beads converts ambient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



This quick and homogenous assay technology provides ample opportunity for integration of laboratory automation. In the development of the following assays the Janus® (PerkinElmer Inc.,) robotic liquid handling instrument was fully implemented to speed development, improve precision and ultimately increase sensitivity of these assays.

3 Material and Methods

Materials – AlphaLISA kits contain 5 components: AlphaLISA Acceptor Beads coated with an anti-analyte antibody, streptavidin-coated donor beads, biotinylated anti-analyte antibody, lyophilized analyte (standard) and 10X AlphaLISA buffer. Assays are performed in white OptiPlate™-384 microplates (PerkinElmer). Initial screening included testing all combinations of 8 antibodies on the donor and/or the acceptor ends, in triplicate, using 3 different concentrations of analyte, blanks and non-targeted IgGs.



Instruments – A Janus® Automated Workstation equipped with WinPREP 4.8 ® software including an 8-tip Varispan™ arm with Versatip® adaptors and Modular Dospense technology arm was used. Plates were read on an Enspire Multimode Reader.

Method with Janus ® liquid handler:

Operator prepares stock solutions of all required reagents (analyte, acceptor beads, biotinylated antibody, donor beads)

↓

Janus® combines 5 µL of analyte with 10 µL of AlphaLISA anti-analyte acceptor beads and 10 µL of biotinylated anti-analyte antibody

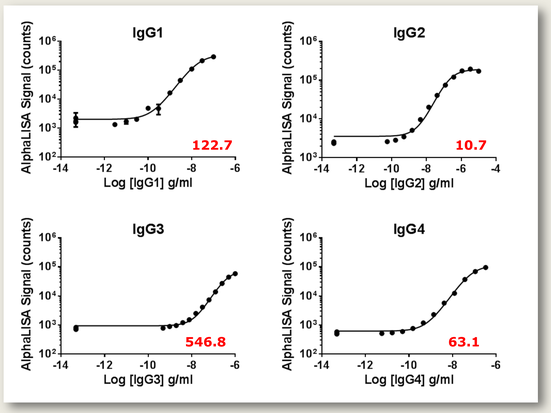
↓ Incubate 60 minutes at 23°C

Janus® adds 25 µL streptavidin alpha donor beads to each well in a single step

↓ Incubate 30 minutes at 23°C in the dark

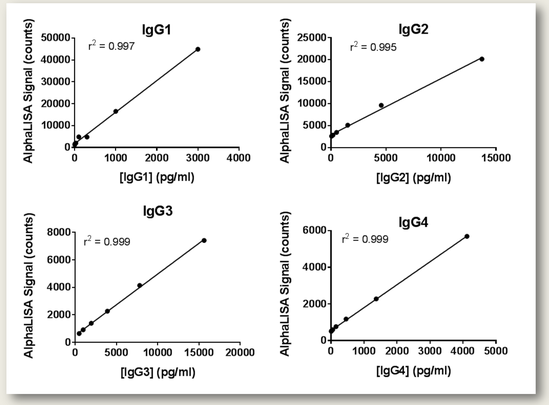
Operator removes completed plate and reads using EnVision® or EnSpire® Multimode Plate Reader and analyzes data using GraphPad Prism®

4 Sensitivity of IgG Isotyping Assays



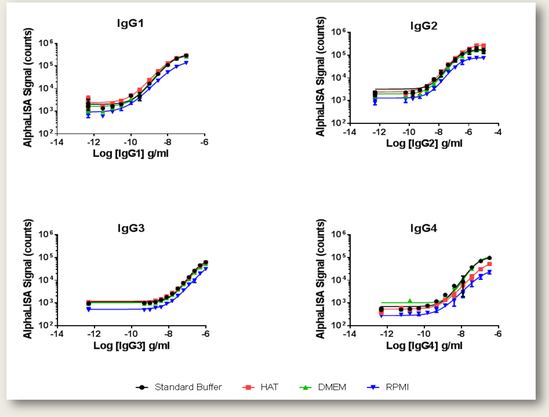
Detection of various IgG subtypes in AlphaLISA buffer. A monoclonal antibody of subtype 1,2,3 or 4 was diluted in AlphaLISA buffer and detected using anti-IgG specific subtype acceptor beads and biotinylated antibodies. Shown in red: Lower detection Limit (pg/ml).

5 Linearity at low analyte concentrations



Linearity of various IgG subtypes in AlphaLISA buffer. Dilution linearity of sample was analyzed on the lower end of the dynamic range for each assay. All assays achieved excellent linearity , indicating the assay will perform well at low ng/ml concentrations of sample.

6 Performance in various cell culture media



Detection of various IgG subtypes in various commonly used cell culture media. A monoclonal antibody of subtype 1,2,3 or 4 was diluted in 3 different cell culture media (HAT, DMEM, RPMI all with 10% FBS) and detected using anti-IgG specific subtype acceptor beads and biotinylated antibodies. In general the media type tested negligibly affects the assay performance with RPMI causing a 2-3 fold increase in EC50 and LDL because of the presence of free biotin.

7 Cross reactivity against other IgG subtypes

Tested against cross reactive analyte \ Assay for	IgG1	IgG2	IgG3	IgG4
IgG1		0%	0.07%	0%
IgG2	5.9%		0%	0%
IgG3	1.0%	0%		0.65%
IgG4	1.4%	0%	0%	

Cross reactivity. Percent cross reactivity against other IgG subtypes determined by spiking 10 ng/ml. Each IgG subtype was tested against the other subtypes (shown on the y axis). Overall, cross reactivity is below 1% for IgG2, IgG3 and IgG4 against the other subtypes tested. IgG1 shows slightly higher cross reactivity for IgG2 ~6%.

8 Summary

The ability to isotype human antibodies at different stages of their development, particularly during clone selection and functional testing, is a decisive key step to achieve successful antibody production showing the right biological functionality. We have developed four highly sensitive kits for detecting human IgG1, 2, 3 and 4, with minimal to no cross reactivity to the other isotypes. In addition, their performance in various cell culture media indicate that these assays are robust and well suited for the detection of these different subtypes in media commonly used in hybridoma production.

Development of these novel assays was greatly facilitated by the use of PerkinElmer's Janus® automated workstation and WinPREP software. Complex and error-prone time-consuming manual processes involving large number of samples were easily and reliably handled with minimal sample volume, providing excellent sensitivity, expanded dynamic range, decreased variability and shorter processing times.

9 References

http://www.perkinelmer.com/pdfs/downloads/AlphaTechCloneSelection.pdf
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