AlphaLISA®: THE Fast and Easy Non-Wash Alternative to ELISA

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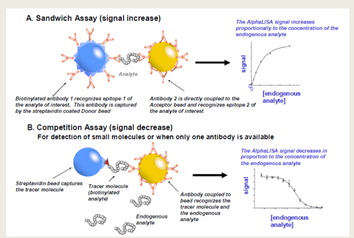
1 Abstract

Although various methods exist for detection and quantification of low analyte concentrations, ELISA is still the most widely adopted method. This non-homogeneous technology offers great selectivity, sensitivity and assay versatility, however has certain limitations like a limited throughput due to wash steps, a generally narrow dynamic range and the incapacity to use low affinity antibodies. The new AlphaLISA® platform does not face those limitations. As it does not require any wash steps, assay development is simple and fast, and hands-on time as well as total assay time are largely reduced. The assays are easy to miniaturize and automate enabling an efficient High Throughput Screening set-up. Like standard ELISA assays, AlphaLISA assays can be designed as either sandwich or competition immunoassays.

Our team successfully developed a multitude of different assays for detection and quantification of analytes from cell culture supernatants, cell lysates and serum/plasma samples. The range of analytes tested includes small molecules (like Substance P) up to large complexes (IgG's to full size phage particles). Excellent performance could be demonstrated with dynamic ranges up to 4.5 log of analyte concentration in the sample, sensitivities below 1 pg/mL with high accuracy and precision. A broad selection of examples, including several biomarkers, will be presented. The results do not only prove that AlphaLISA can be used universally instead of ELISA, but also that AlphaLISA is an enabling technology for applications where measuring low affinity interactions between two or more binding partners is required. This, together with its ease of use, makes AlphaLISA THE alternative generic technology platform replacing ELISA..

2 AlphaScreen® Immunoassay Formats Using AlphaLISA Beads



3 Human Insulin Quantification in Serum

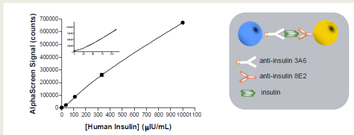


Figure 1. Human insulin calibration curve. The assay was performed in a 384-well microplate in a 50 µL total volume using 5 µL insulin calibrators prepared in insulin-depleted serum. Final concentrations of reagents in well were 1 nM biotinylated 3A6 antibody, 10 µg/mL 8E2 antibody-conjugated AlphaLISA Acceptor beads, and 40 µg/mL streptavidin-Donor beads. Assay buffer composition was 25 mM Hepes pH 7.4, 1 µg/mL Dextran T-500, 0.5% Triton X-100, and 0.1% casein. A detection limit value of 2 µIU/mL (85 pg/mL) and a dynamic range of 2-3,000 µIU/mL were obtained at 1-hour incubation time after bead addition.

4 Detection of Human IgG from Cell Culture Supernatants

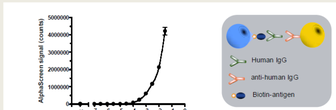


Figure 2. Human IgG calibration curve. The assay was performed in a 384-well microplate in a 50 µL total volume using 5 µL human IgG standards diluted in DMEM/F12 + 10% FBS. Final concentrations of reagents in well were 1 µg/mL biotinylated antigen, 20 µg/mL anti-human IgG antibody-conjugated AlphaLISA Acceptor beads, and 20 µg/mL streptavidin-Donor beads. Assay buffer composition was 25 mM Hepes pH 7.4, 1 mg/mL Dextran T-500, 0.5% Triton X-100, and 0.1% casein. The assay presented a sensitivity of 3.6 ng/mL and a dynamic range of 4 log units.

5 Quantification of Amyloid Beta 1-40 Peptide (Aβ40) in Cell Culture Supernatants

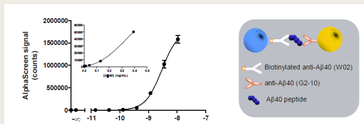


Figure 3. Human Aβ40 calibration curve. The assay was performed in a 384-well microplate in a 50 µL total volume using 30 µL Aβ40 standards. The biotinylated W02 anti-Aβ40 antibody was used at a final dilution of 1:1,500. G2-10 anti-Aβ40 antibody-conjugated AlphaLISA Acceptor beads and streptavidin-Donor beads were used at final concentrations of 10 and 40 µg/mL, respectively. Assay buffer was 30 mM Tris-HCl pH 7.4, 0.02% Tween-20 and 0.02% casein. The assay presented a sensitivity of 5 pg/mL and a dynamic range of 3.5 log units.

6 Automation of Amyloid Beta 1-40 Assay using the Janus® Automated Workstation

	Plate 1		Plate 2		Plate 3		Plate 4	
	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B	Sample A	Sample B
Average signal (counts)	1030240	1442643	980384	1450161	950908	1357994	900625	1302525
Standard Deviation	23614	27870	26651	121413	37322	35602	49624	49878
S/B ratio	554	776	550	814	522	746	537	777
%CV	2.3	1.9	2.7	8.3	3.9	2.6	5.5	3.8
Z' value	0.92	0.93	0.91	0.74	0.87	0.91	0.83	0.88

Table 1. Robustness of the automated Aβ40 AlphaLISA assay using the Janus™ Automated Workstation from PerkinElmer. Four 384-well microplates were assayed with two cell culture supernatant samples (A and B). Excellent assay reproducibility with Z' values ranging between 0.74 and 0.93 were obtained.

7 Human TNFα Immunoassay

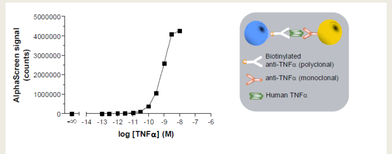


Figure 4. TNFα calibration curve. The assay was performed in a 384-well microplate in a 25 µL total volume using 5 µL TNFα dilutions. Final concentrations of reagents in well were 1 nM biotinylated anti-TNFα, 20 µg/mL anti-TNFα antibody-conjugated AlphaLISA Acceptor beads, and 20 µg/mL streptavidin-Donor beads. Assay buffer was 25 mM Hepes pH 7.4, 100 mM NaCl, 0.1% Tween-20. The assay presented a high sensitivity (1 pM, 18 pg/mL) and a wide dynamic range of 3.5 log units.

8 Measurement of CHOP (contaminant CHO Host Cell Proteins) in Cell Lysates

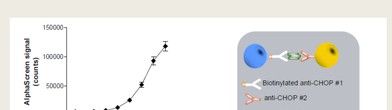


Figure 5. Titration of CHOP. The anti-CHOP antibodies used in this assay are goat polyclonal antibodies developed to recognize more than 40 proteins from CHO cells. The assay was performed in a 384-well microplate in a 30 µL total volume using 5 µL CHOP dilutions. Final concentrations of reagents in well were 3 nM biotinylated anti-CHOP, 10 µg/mL anti-CHOP antibody-conjugated AlphaLISA Acceptor beads, and 20 µg/mL streptavidin-Donor beads. Assay buffer was 25 mM Hepes pH 7.4, 0.5% Triton X-100 and 0.33% casein. The assay presented a sensitivity of 19 ng/mL and a dynamic range of 2-3 log units after an overnight incubation.

9 Detection of Bulky Protein Chondroitin Sulphate Proteoglycan

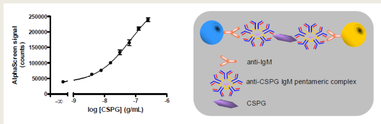


Figure 6. Titration of chondroitin sulphate proteoglycan (CSPG). In this example, CSPG was indirectly detected by anti IgM antibodies conjugated to AlphaLISA beads recognizing the pentameric anti-CSPG IgM molecule (directed against the glycosaminoglycans repeats). The assay was performed in a 384-well microplate in a 25 µL total volume using 5 µL CSPG dilutions. Final concentrations of reagents in well were 3.6 µg/mL anti-CSPG IgM, and 20 µg/mL of both anti-IgM AlphaLISA Acceptor beads and anti-IgM -Donor beads. Assay buffer was 25 mM Hepes pH 7.4, 1 mg/mL Dextran T-500, 0.5% Triton X-100 and 0.1% casein. The dose-dependent signal increase obtained demonstrates the possibility of using large IgM molecules in AlphaLISA, and the ability of detecting large glycosylated proteins.

10 Detection of M13 Phage Particles (1 µm in size)

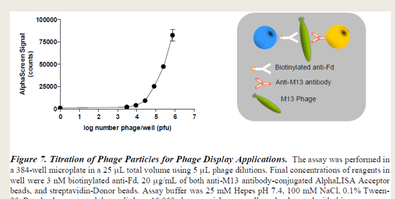


Figure 7. Titration of Phage Particles for Phage Display Applications. The assay was performed in a 384-well microplate in a 25 µL total volume using 5 µL phage dilutions. Final concentrations of reagents in well were 3 nM biotinylated anti-Fd, 20 µg/mL of both anti-M13 antibody-conjugated AlphaLISA Acceptor beads, and streptavidin-Donor beads. Assay buffer was 25 mM Hepes pH 7.4, 100 mM NaCl, 0.1% Tween-20. Results demonstrated that as little as 10,000 phage particles per well can be detected with this assay.

11 Detection of Substance P Polypeptide Competition Assay

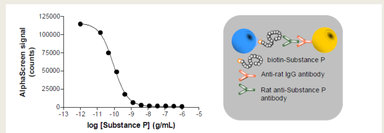


Figure 8. Calibration curve of Human Substance P (11-amino acid polypeptide). The assay was performed in a 384-well microplate in a 50 µL total volume using 20 µL Substance P dilutions. Final concentrations of reagents in well were 0.1 nM biotinylated Substance P, rat anti-substance P antibody diluted 1:3,000, 10 µg/mL anti-rat IgG antibody-conjugated AlphaLISA Acceptor beads, and 40 µg/mL streptavidin-Donor beads. Assay buffer composition was 30 mM Tris-HCl pH 7.4, 0.02% Tween-20 and 0.02% casein. A detection limit value of 9 pg/mL was obtained with a dynamic range of 2 log units.

12 Conclusion

AlphaLISA is a new ELISA replacement platform, perfectly suited for the detection and quantification of a wide variety of analytes based on sandwich or competitive immunoassays.

AlphaLISA brings all the following advantages:

- Homogeneous no wash technology: easily miniaturized and automated
- Wide dynamic range: 3 or 4 log units in most standard assays minimizing dilution steps
- Highly sensitive: signal amplification ensures the utmost sensitivity for the detection of low concentration analytes.
- Broad range of affinities: allows the use of high and low affinity antibodies
- Broad range of sizes of analyte: from small hormones to large bulky Complexes

AlphaLISA Custom Assay Development Services available.

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