Automation and Miniaturization of Immunoassays for Drug Discovery: AlphaLISA™, a Sensitive No-Wash Assay

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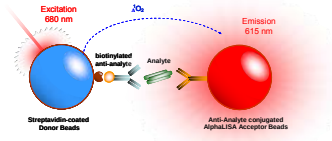
1 Introduction

AlphaLISA™, an immunoassay for the detection of various analytes and biomarkers, offers many advantages over ELISA-like technologies. AlphaLISA is homogeneous, thus not requiring a single wash step. Samples can be tested without dilution due to the large dynamic range (approximately 3 – 5 logs). AlphaLISA assays are simple to develop, rapid to perform as well as sensitive. The technology allows working with small volumes (1 – 5 µL) and is therefore easy to miniaturize and automate enabling High Throughput Screening (HTS).

Quantitative detection of insulin in various 96-, 384- and 1536-well microplates was performed with final assay volumes ranging from 10 – 50 µL. Lower detection limits (LDL), EC_{50} values and maximum counts were compared. Also, Insulin, EPO, VEGF and IgG assays were fully automated using the JANUS® Automated Workstation with very low variation and assay parameters similar to the manual mode. Excellent sensitivities were obtained demonstrating how easy and simple miniaturization and automation are using AlphaLISA.

2 AlphaLISA: Principle



The biotinylated anti-analyte antibody binds to the Streptavidin-coated 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 excitation of the donor beads provokes the release of singlet oxygen molecules that triggers a cascade of energy transfer to the acceptor beads resulting in a sharp peak of light emission at 615 nm.

3 Plate Comparison: Plates

PKI Plates and Assay setup

Plate	PKI Cat#	Color	Assay volume	Protocol
OptiPlate™-96	6005290	white	50 µL	A
½ AreaPlate-96	6005560	white	50 µL	A
ProxiPlate™-96	6006290	white	50 µL	A
OptiPlate™-384	6007290	white	50 µL	A
AlphaPlate™-384	6005350	light-gray	50 µL	A
ProxiPlate™-384 Plus	6008280	white	20 µL	B
ProxiPlate™-384 (light-gray)	custom	light-gray	20 µL	B
AlphaPlate™-1536 *	6004350	light-gray	10 µL	C

* White OptiPlates-1536 are not recommended in AlphaLISA assays with high counts due to higher crosstalk compared to light-gray AlphaPlate-1536

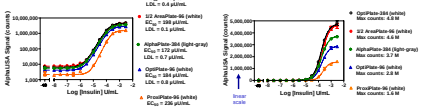
4 Plate Comparison: Protocols

Insulin AlphaLISA Assay - Protocols

Step	Action	A	B	C1	C2
1	Insulin standard dilutions	5 µL	5 µL	1 µL	5 µL
2	Mix: anti-insulin acceptor beads (10 µg/mL final) + biotinylated anti-insulin antibody (1 nM final)	20 µL	5 µL	4 µL	2.5 µL
3	Incubation (RT)	60 min			
4	Streptavidin donor beads	25 µL	10 µL	5 µL	2.5 µL
5	Incubation (RT, dark)	30 min			
6	Reading	EnVision® instrument			

5 Plate Comparison: Insulin Assay

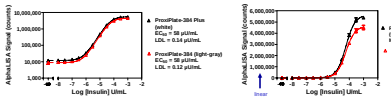
Protocol A: 50 µL



► similar EC_{50} and LDL for all plates except ProxiPlate-96 (less sensitive for AlphaLISA, not recommended)
► counts proportional to liquid height:
→ similar high count levels for Optiplate-384, ½ Areaplate-96
→ lowest counts for Optiplate-96, ProxiPlate-96
→ lower counts for AlphaPlate-384 compared to OptiPlate-384 due to light-gray color, but similar LDL, EC_{50} (note: light-gray color reduces cross-talk)

6 Plate Comparison: Insulin Assay

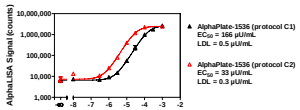
Protocol B: 20 µL



► similar EC_{50} and LDL for white and light-gray ProxiPlate-384
► more sensitive than 50-µL assay due to higher final analyte concentration
► lower counts for light-gray (custom) compared to white ProxiPlate-384 due to color, but similar LDL, EC_{50} (note: light-gray color reduces cross-talk)

7 Assay Miniaturization: Insulin Assay

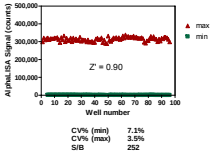
Protocol C: 10 µL assays with 1 or 5 µL of sample



► similar maximum counts obtained for both protocols
► as expected, protocol C2 (5 µL insulin standard) results in a better sensitivity compared to protocol C1 (1 µL)

8 Assay Miniaturization: Z'-Factor

Insulin AlphaLISA Assay, AlphaPlate-1536



► low CV (≤ 7 %) and high Z' value (0.9) obtained
► light-gray AlphaPlate-1536 is recommended for insulin AlphaLISA assay

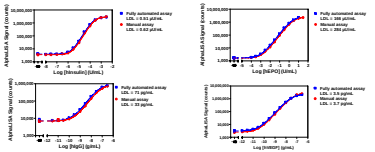
9 Automation: JANUS® Workstation

The JANUS® is a robotic liquid handling system designed for the efficient automation of sample preparation procedures:
→ liquid transfers from any combination of laboratory container including 384-well, 1536-well plates possible
→ suited for dispensing into 96-, 384- and 1536-well plates

AlphaLISA assay automation:
→ JANUS® workstation controlled using the WinPREP® 4.1 software
→ all serial dilutions of the standard curve and reagent dispensing in the 384-well OptiPlate carried out using the Varispan™ (8-tip dispensing arm)
→ JANUS® deck protected from light while dispensing the donor beads
→ all steps of the manual assay mimicked during automation (e.g. tip pre-wetting, tip change) and same protocol and reagents used for the automated and manual assays

10 Automation: Manual vs. JANUS®

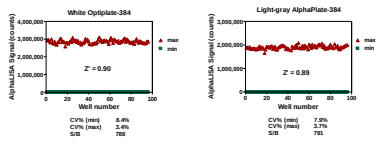
hInsulin, hEPO, hIgG, hVEGF (Optiplate-384, 50 µL)



► similar LDL obtained for all assays in automatic versus manual mode

11 Automation: Z'-Factor

JANUS® Workstation, OptiPlate-384



► low CV (≤ 8 %) and high Z'-value (0.9) obtained for both plates
► lower counts obtained with light-gray AlphaPlate, but similar S/B ratios
► both plates, white OptiPlate-384 and light-gray AlphaPlate-384 are suited for insulin AlphaLISA assay

12 Conclusions

➢ **AlphaLISA is the easiest ELISA technology ever and offers the following advantages**:
* Detection of molecules of interest in a highly sensitive, quantitative, reproducible and user-friendly manner.
* No wash step required, low sample volumes: easy to miniaturize and automate using the JANUS® workstation as demonstrated with four different AlphaLISA assays.

➢ **AlphaLISA can be performed using various plate formats**:
* 96-well format: ½AreaPlate-96 recommended
* 384-well format: all plates perform well, light-gray AlphaPlate-384 recommended for high counts due to cross-talk reduction
* 1536-well format: light-gray AlphaPlate-1536 recommended