DEVELOPMENT OF NEW ALPHALISA NO-WASH IMMUNOASSAY KITS FOR SENSITIVE, RAPID AND EFFICIENT QUANTIFICATION OF CYTOKINES

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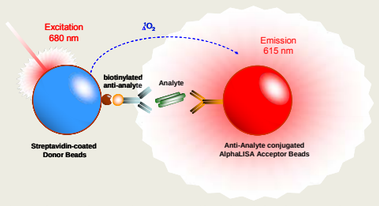
1 Introduction

Enzyme-linked Immunosorbent Assay (ELISA) is the most widely adopted method for detection and quantification of cytokines and other biomarkers. This traditional technology offers good selectivity, sensitivity and assay versatility; however, it has certain disadvantages such as limited dynamic range and low throughput due to the numerous wash steps. In addition, ELISA is not well suited for the use of medium or low affinity antibodies. In contrast, chemiluminescent bead-based AlphaLISA® assays do not face these limitations. AlphaLISA dynamic range is generally between 3 to 4.5 log units and the absence of wash steps allows performing these assays in high throughput mode. Assay development is simple and fast, and hands-on time as well as total assay time is significantly reduced. AlphaLISA assays are easy to miniaturize and automate enabling both Research and High Throughput Screening (HTS) laboratories to efficiently quantify analytes of interest. PerkinElmer has developed several AlphaLISA assays for the detection and quantification of cytokines in cell culture supernatants and serum samples. Experiments showing Lower Detection Limit (LDL) and dynamic range will be presented. The performance of the assays is excellent, including a large dynamic range, high sensitivity, accuracy and precision. The overall results confirm the user-friendly AlphaLISA technology as a new generation of tools available for immunoassays.

No-wash AlphaLISA bead-based Immunoassay:



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. Excitation of the Donor Beads provokes the release of singlet oxygen molecules that triggers a cascade of energy transfers within the Acceptor Beads resulting in a sharp peak of light emission at 615 nm.

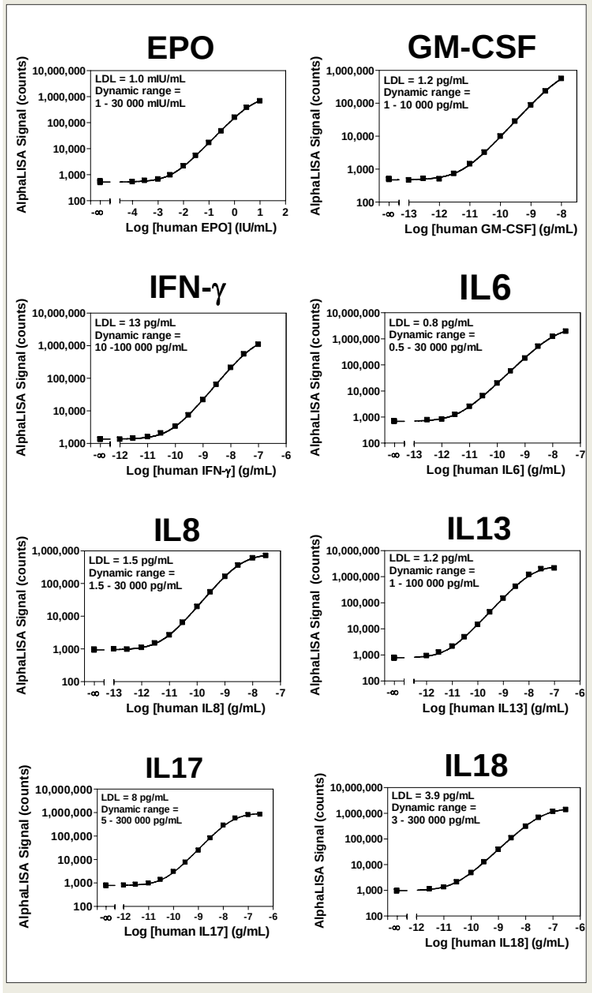
2 Materials and Methods

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 and 10X AlphaLISA Buffer. Assays are performed in white OptiPlate™-384 microplates (PerkinElmer).

Typical Protocol (Total volume: 50 µL; assay time: 90 minutes):

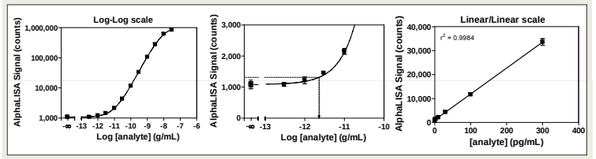
Add 5 µL of each analyte standard dilution (or sample)
Add 20 µL of a 2.5X Mix: AlphaLISA Anti-Analyte Acceptor beads (10 µg/mL final) and Biotinylated Antibody Anti-Analyte (1 nM final)
↓ Incubate 60 minutes at 23˚C
Add 25 µL 2X SA-Donor beads (40 µg/mL final)
↓ Incubate 30 minutes at 23˚C in the dark
Read using EnVision®-Alpha Reader (PerkinElmer LAS, Inc.)

3 AlphaLISA Cytokine Immunoassay Kits



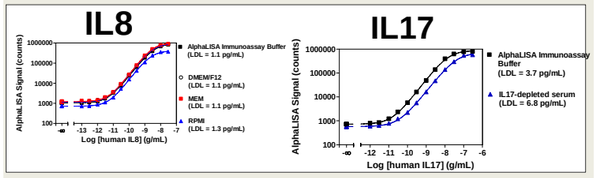
►Assays were performed in either AlphaLISA HiBlock (IFN-γ) or Immunoassay Buffer (all others). For each kit, a low LDL and wide dynamic range were obtained.

4 Data analysis



► Data are analyzed using non-linear regression fit.
► Non-linear fit using 4-parameter logistic equation (sigmoidal dose-response curve with variable slope) and a 1/Y2 data weighting yield the most precise values and the widest dynamic range.
► The Lower Detection Limit (LDL) is calculated by interpolating the average background counts (12 wells without analyte) + 3 × the standard deviation value (average background counts + (3 × SD)) on the standard curve.

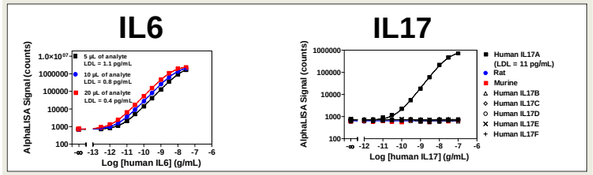
5 Using Cell Culture Media and Serum



► AlphaLISA assays can be performed in various cell culture media even though some of the media contain biotin (e.g. RPMI).

► AlphaLISA assays can be performed in serum with limited effect on maximum counts and Lower Detection Limit.

6 Sensitivity and Selectivity



► AlphaLISA assays can be performed with 5 µL sample volume (regular sample volume) or higher sample volume (e.g. 10 µL or 20 µL) to increase sensitivity.

► No cross-reactivity observed up to 100 ng/mL of rat or murine IL17A, or other human IL17 isoforms.

7 Reproducibility (VEGF kit)

Sample (pg/mL)	% CV				Recovery (in Buffer)	Recovery (in Serum)
	Intra-assay precision	Inter-assay precision	Inter-lot variability	Intra-lot reproducibility		
3000	5.5	7.9	3.2	8.1	106%	101%
300	4.0	8.1	2.7	11.1	110%	99%

►The intra-assay precision is calculated in one assay using nine replicates of each sample.
►The inter-assay precision is calculated in three independent assays using nine replicates of each sample.
►The inter-lot variability is calculated in one assay using three independent lots of reagents in triplicates.
►The intra-lot reproducibility is calculated using nine independent assays done by three experimenters.
►The percentage of recovery of three known concentrations of analyte is calculated by comparing the measured versus the theoretical amount for each concentration in nine independent assays.

8 Summary and Conclusions

AlphaLISA is THE new ELISA replacement platform with:

✓No-wash steps (homogeneous assays)
✓Quick assays (2 steps in 90 minutes)
✓Low sample volume needed (5 µL of analyte in 50 µL total assay volume)
✓Wide dynamic range
✓High sensitivity
✓Possibility of miniaturization (from 96 to 384 and 1536-well plate format)
✓Multiple medium compatibility such as serum, cell culture media and AlphaLISA buffer

Selection of AlphaLISA Cytokine Kits (500 and 5,000 assay points):

Analyte:	Cat #:	Analyte:	Cat #:
VEGF(May 08)	AL201	IL6 (Dec 08)	AL223
EPO (May 08)	AL206	IL8 (Dec 08)	AL224
GM-CSF (Dec 08)	AL216	IL17 (Dec 08)	AL219
IFN-g (Dec 08)	AL217	IL13 (April 09)	Coming soon
		IL18 (April 09)	Coming soon

Many other kits are available or coming soon !

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