Development of 8 New No-Wash Immunoassay Kits for the Detection of VEGF, Amyloid ß1-40, Amyloid ß1-42, Insulin, Human IgG, EPO, HIV p24 and TNFα Using PerkinElmer AlphaLISA™

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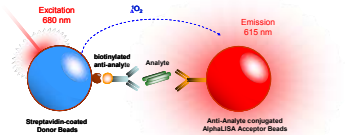
1 Abstract

ELISA is the most widely adopted method for detection and quantification of low analyte concentrations. This traditional technology offers good selectivity, sensitivity and assay versatility; however, it has certain disadvantages such as limited dynamic range and limited throughput. In addition, the numerous required wash-steps limit its ability to measure low affinity antibodies. The PerkinElmer AlphaLISA™ platform does not face these limitations. It does not require any wash steps. Assay development is simple and fast and hands-on time as well as total assay time are significantly reduced. These low variability assays are easy to miniaturize and automate enabling an efficient High Throughput Screening set-up.

VEGF, Amyloid ß1-40, Amyloid ß1-42, Insulin, human IgG, EPO, HIV p24 and TNFα AlphaLISA assays were fully developed for detection and quantification in cell culture supernatants and serum samples. Standard curves showing Lower Detection Limit (LDL) and dynamic range will be presented for each analyte as well as assay variability results. Excellent performance is demonstrated with large dynamic ranges, high sensitivities, high accuracy and precision. The overall results confirm the user-friendly AlphaLISA technology as a new generation of tools available for immunoassays. Optimized assays for the listed analytes were converted to kit form to increase the accessibility of AlphaLISA technology to more researchers.

2 AlphaScreen® Immunoassay Format Using AlphaLISA Beads



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 Materials and Methods

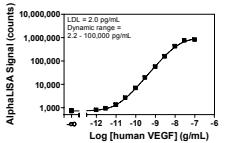
Materials – The AlphaLISA kit contains 5 components: AlphaLISA Acceptor Beads coated with an Anti-Analyte Antibody, Streptavidin-coated Donor Beads, Biotinylated Anti-Analyte Antibody, lyophilized analyte and 10X AlphaLISA ImmunoAssay Buffer.

General assay procedure - The assays were performed in White OptiPlate™-384 microplates from PerkinElmer. All assay incubations were performed at 23ºC and the microplates were sealed with TopSeal-A™ (PerkinElmer) during that time. The fluorescence signals were read using an excitation filter of 680 nm and an emission filter of 615 nm on an EnVision® Multilabel Reader (PerkinElmer).

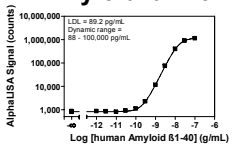
Typical Protocol - Add in triplicate: 5 µL of standard (analyte) in a range of concentrations and 20 µL of a mix of AlphaLISA Acceptor Beads/Biotinylated Antibody (10 µg/mL / 1 nM final in the well). Incubate 60 minutes at 23°C and add 25 µL of Streptavidin Donor Beads (40 µg/mL). Incubate 30 minutes at 23°C protected from light and read on EnVision-Multilabel Reader (total volume: 50 µL; time of the assay: 90 minutes).

4 The 8 New No-Wash Immunoassay Kits

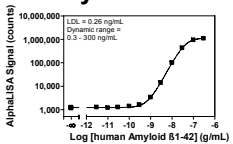
VEGF



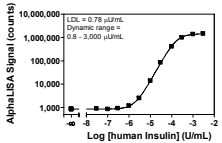
Amyloid ß1-40



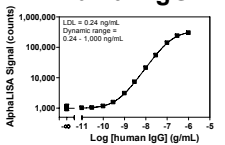
Amyloid ß1-42



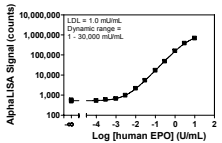
Insulin



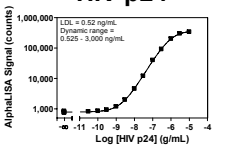
Human IgG



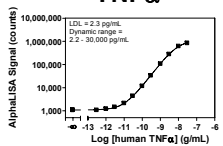
EPO



HIV p24

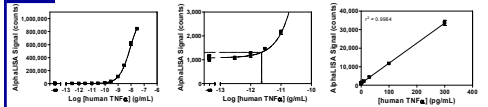


TNFα



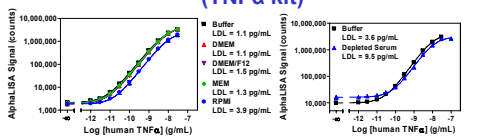
► For each kit a low LDL and a wide dynamic range has been obtained (dilution of analytes in AlphaLISA ImmunoAssay Buffer).

5 Data Analysis (TNFα kit)



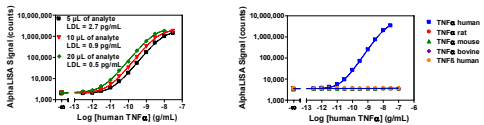
- ► Data can be analyzed using linear or non-linear regression fit.
- ► Non-linear fit using 4-parameter logistic equation (sigmoidal dose-response curve with variable slope) and a $1/Y^2$ data weighting yield the most **precise** values and the widest dynamic range.
- ► Values at concentrations of analyte above the hook point must be removed for correct analysis.
- ► The Lower Detection Limit (LDL) is calculated by interpolating on the standard curve the background value + 3 standard deviations.

6 Using Cell Culture Media and Serum (TNFα kit)



- ► The assay can be performed in various cell culture media even though some of the media contain competing biotin (e.g. RPMI).
- ► The assay can be performed in serum with limited effect on the Lower Detection Limit.

7 Sensitivity and Selectivity (TNFα kit)



- ► Effect of **analyte volume** on the TNFα assay in 50 µL (dilution of analytes in AlphaLISA ImmunoAssay buffer).
- ► Want more sensitivity? Increase the sample volume.
- ► No cross-reactivity observed up to 100 ng/mL of human TNFß, rat, mouse or bovine TNFα.

8 Reproducibility (TNFα kit)

Sample (pg/mL)	% CV				Recovery
	Intra-assay precision	Inter-assay precision	Inter-lot variability	Intra-lot reproducibility	
3,000	4.8	6.5	4.8	4.9	111%
300	9.0	6.8	4.0	10.2	102%
30	5.0	7.1	6.3	9.1	113%

- ► The intra-assay precision is calculated in one assay using nine replicates of each samples.
- ► 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.

9 Summary and Conclusions

- ► AlphaLISA is THE new ELISA replacement platform with:
  - ✓No-wash steps (homogeneous assays)
  - ✓Wide dynamic range
  - ✓High sensitivity
- ► Miniaturization is possible with this technology
- ► With these new kits, the AlphaLISA technology is even more accessible
  - ✓Low cost
  - ✓Quick assays (2 steps in 90 minutes)
  - ✓Low sample volume needed (5 µL of analyte in 50 µL total volume)
  - ✓Multiple medium compatibility such as serum, cell culture media and AlphaLISA buffer

10 Kits Available

Catalog numbers

Analytes	500 Assay Points	5,000 Assay Points
VEGF	AL201C	AL201F
Aß1-40	AL202C	AL202F
Aß1-42	AL203C	AL203F
Insulin	AL204C	AL204F
Human IgG	AL205C	AL205F
EPO	AL206C	AL206F
HIV p24	AL207C	AL207F
TNFα	AL208C	AL208F

Many other kits are coming soon!