TECHNICAL NOTE

AlphaLISA Technology

# AlphaLISA Assays for Immune Checkpoint Detection and Quantitation

**Author:**

**Jun Zhang**

PerkinElmer, Inc.
Hopkinton, MA

## Introduction

Immune checkpoints are molecular markers that can regulate an immune system attack on cancer cells, and they have become highly regarded as promising candidates for immunotherapies. These molecules can be either functional stimulatory (e.g. CD28) or inhibitory checkpoint molecules (e.g. CD276, CD272, CTLA-4, LAG3, PD-1, TIM-3 and VISTA). AlphaLISA biomarker detection kits are designed to detect and quantify the levels of these molecules in cell culture media, serum and cell lysates.

AlphaLISA® technology allows for the detection and quantitation of these immune checkpoints in buffer, cell culture media, serum, and cell lysates in a highly sensitive, reproducible and user-friendly way. In an AlphaLISA assay, a 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 excitation of the Donor beads provokes the release of singlet oxygen molecules that triggers a cascade of energy transfer within the Acceptor beads, resulting in a sharp peak of light emission at 615 nm (Figure 1). This light emission can then be detected on an Alpha-enabled reader, such as the EnVision® multilabel plate reader.

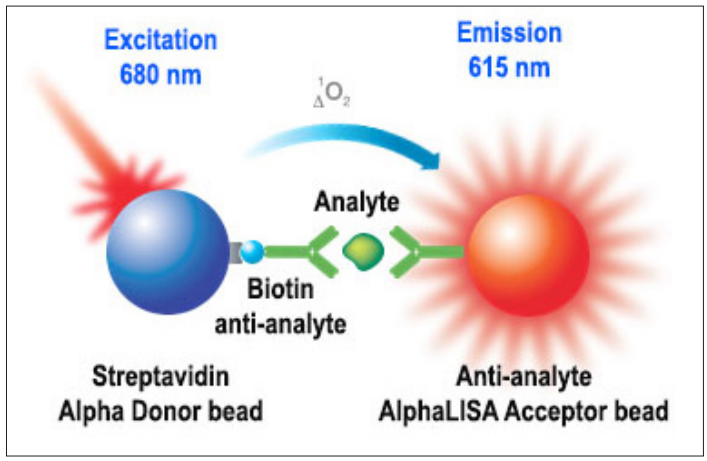


*Figure 1.* Assay principle for AlphaLISA detection assay.



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## Experimental Protocol

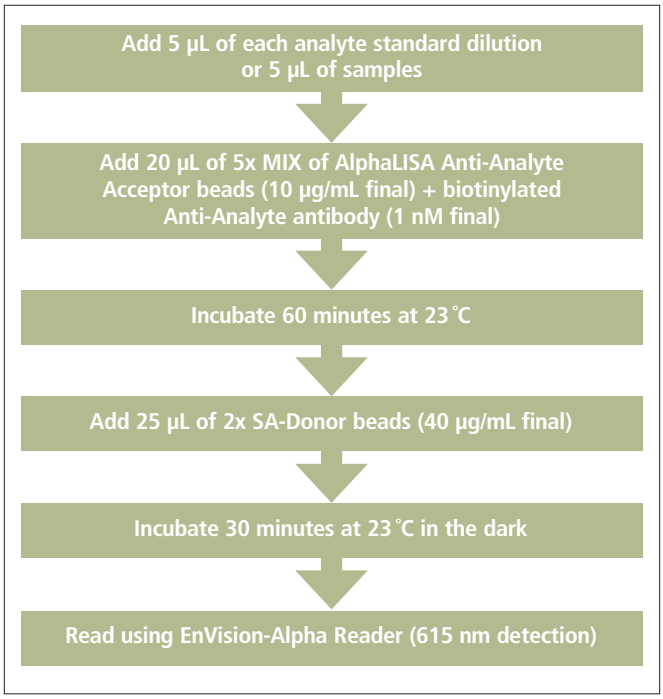


*Figure 2.* General workflow for an AlphaLISA detection assay. Procedures may vary slightly for each individual kit.

## Standard Curves

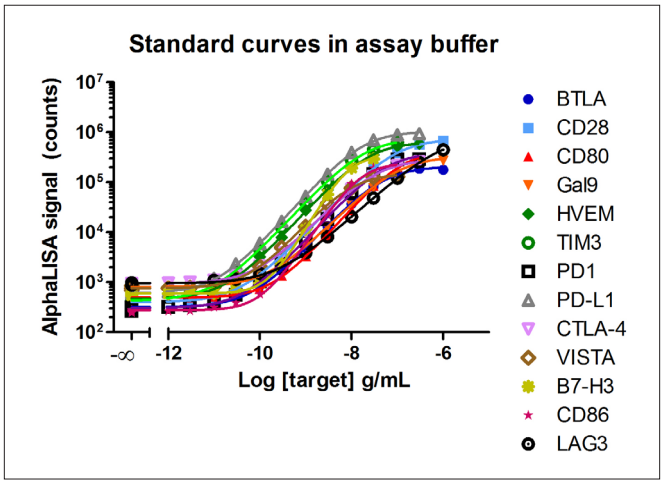


*Figure 3.* Standard curves for AlphaLISA immune checkpoint detection assays. AlphaLISA assays provide high signal-to-background and high sensitivity with broad dynamic range.

## Assay Performance

*Table 1.* Assay performance for AlphaLISA kits.

| Target | Part Number | LDL[1] | LLOQ[2] | Dynamic Range | %CV[3] (Intra) | %CV[3] (Inter) |
|---|---|---|---|---|---|---|
| B7-H3 (CD276) | AL3060 | 42 pg/mL | 104 pg/mL | 42 - 100,000 pg/mL | 5 | 7 |
| BTLA (CD272) | AL3062 | 11 pg/mL | 38 pg/mL | 11 - 300,000 pg/mL | 6 | 10 |
| CTLA-4 | AL3050 | 34 pg/mL | 121 pg/mL | 34 - 100,000 pg/mL | 4 | 7 |
| CD28 | AL3044 | 9 pg/mL | 26 pg/mL | 9 - 1,000,000 pg/mL | 3 | 4 |
| CD86 | AL3045 | 25 pg/mL | 75 pg/mL | 25 - 100,000 pg/mL | 4 | 5 |
| CD80 | AL3055 | 36 pg/mL | 126 pg/mL | 36 - 300,000 pg/mL | 6 | 8 |
| GAL9 (Galectin) | AL3051 | 9 pg/mL | 42 pg/mL | 9 - 1,000,000 pg/mL | 5 | 10 |
| HLA-C | AL3061 | 56 pg/mL | 193 pg/mL | 56 - 300,000 pg/mL | 4 | 5 |
| HLA-E | AL3057 | 76 pg/mL | 326 pg/mL | 76 - 3,000,000 pg/mL | 4 | 11 |
| HVEM | AL3054 | 3 pg/mL | 11 pg/mL | 3 - 300,000 pg/mL | 3 | 8 |
| LAG-3 | AL3058 | 74.1 pg/mL | 331.1 pg/mL | 74 - 1,000,000 pg/mL | 7 | 13 |
| PD-1 | AL343 | 9 pg/mL | 37 pg/mL | 9 - 100,000 pg/mL | 5 | 15 |
| PD-L1 | AL355 | 2 pg/mL | 10 pg/mL | 2 - 300,000 pg/mL | 4 | 6 |
| TIM-3 | AL3052 | 2.5 pg/mL | 8 pg/mL | 2.5 - 100,000 pg/mL | 6 | 6 |
| VISTA | AL3059 | 14.2 pg/mL | 56.6 pg/mL | 14.2 - 100,000 pg/mL | 5 | 9 |

[1] The LDL is calculated by interpolating the average background counts (12 wells without analyte) + 3 x standard deviation value (average background counts + (3xSD)) on the standard curve.

[2] The LLOQ as measured here is calculated by interpolating the average background counts (12 wells without analyte) + 10 x standard deviation value (average background counts + (10xSD)) on the standard curve.

[3] % CV data were calculated using two different kit lots. For each lot, the standard curves were prepared in kit assay buffer. Each assay consisted of one standard curve comprising 12 data points (each in triplicate) and 12 background wells (no analytes). The assays were performed in 384-well format. The intra-assay precision was determined using a total of seven independent determinations in triplicate, shown as CV%. The inter-assay precision was determined using a total of seven independent determinations with 21 measurements, shown as CV%.

## Validated Sample Matrices

*Table 2.* Validated sample matrices for AlphaLISA immune checkpoint detection assays.

| Target | Part Number | Buffer | DMEM | RPMI | Serum |
|---|---|---|---|---|---|
| B7-H3 (CD276) | AL3060 | ✓ | ✓ | ✓ | ✓ |
| BTLA (CD272) | AL3062 | ✓ | ✓ | Not recommended | ✓ |
| CTLA-4 | AL3050 | ✓ | ✓ | ✓ | ✓ |
| CD28 | AL3044 | ✓ | ✓ | ✓ | Not recommended |
| CD86 | AL3045 | ✓ | ✓ | Not recommended | ✓ |
| CD80 | AL3055 | ✓ | ✓ | Not recommended | ✓ |
| GAL9 (Galectin) | AL3051 | ✓ | ✓ | Not recommended | ✓ |
| HLA-C | AL3061 | ✓ | ✓ | Not recommended | Not recommended |
| HLA-E | AL3057 | ✓ | ✓ | Not recommended | Not recommended |
| HVEM | AL3054 | ✓ | ✓ | Not recommended | ✓ |
| LAG-3 | AL3058 | ✓ | ✓ | ✓ | ✓ |
| PD-1 | AL343 | ✓ | ✓ | ✓ | ✓ |
| PD-L1 | AL355 | ✓ | ✓ | ✓ | ✓ |
| TIM-3 | AL3052 | ✓ | ✓ | ✓ | ✓ |
| VISTA | AL3059 | ✓ | ✓ | Not recommended | ✓ |

## Instrumentation

All immune checkpoint AlphaLISA kits were developed using the EnVision multilabel plate reader. In addition to fast, sensitive Alpha technology detection, the EnVision plate reader provides fluorescence intensity, luminescence, absorbance, fluorescence polarization, and time-resolved fluorescence detection technologies. The system is based on hybrid technology, combining filters and a monochromator for enhanced flexibility. It incorporates proprietary Direct Double Optics™ technology for high speed and sensitivity in simultaneous filter-based readouts, such as FRET assays, and for TRF, lamp-based excitation is standard, with the option of a high energy laser for higher speed and sensitivity.

*Figure 4.* EnVision multilabel plate reader.

## Products and Part Numbers

*Table 3.* Products for AlphaLISA immune checkpoint detection assays.

| PerkinElmer Product | Format | Catalog Number |
|---|---|---|
| B7-H3 (CD276) (human) AlphaLISA Detection Kit | 500 assay points | AL3060C |
| | 5000 assay points | AL3060F |
| BTLA (CD272) (human) AlphaLISA Detection Kit | 500 assay points | AL3062C |
| | 5000 assay points | AL3062F |
| CD28 (human) AlphaLISA Detection Kit | 500 assay points | AL3044C |
| | 5000 assay points | AL3044F |
| CD80 (human) AlphaLISA Detection Kit | 500 assay points | AL3055C |
| | 5000 assay points | AL3055F |
| CD86 (human) AlphaLISA Detection Kit | 500 assay points | AL3045C |
| | 5000 assay points | AL3045F |
| CTLA-4 (human) AlphaLISA Detection Kit | 500 assay points | AL3050C |
| | 5000 assay points | AL3050F |
| GAL9 (Galectin) (human) AlphaLISA Detection Kit | 500 assay points | AL3051C |
| | 5000 assay points | AL3051F |
| HLA-C (human) AlphaLISA Detection Kit | 500 assay points | AL3061C |
| | 5000 assay points | AL3061F |
| HLA-E (human) AlphaLISA Detection Kit | 500 assay points | AL3057C |
| | 5000 assay points | AL3057F |
| HVEM (human) AlphaLISA Detection Kit | 500 assay points | AL3054C |
| | 5000 assay points | AL3054F |
| LAG-3 (human) AlphaLISA Detection Kit | 500 assay points | AL3058C |
| | 5000 assay points | AL3058F |
| PD-1 (human) AlphaLISA Detection Kit | 500 assay points | AL343C |
| | 5000 assay points | AL343F |
| PD-L1 (human) AlphaLISA Detection Kit | 500 assay points | AL355C |
| | 5000 assay points | AL355F |
| TIM-3 (human) AlphaLISA Detection Kit | 500 assay points | AL3052C |
| | 5000 assay points | AL3052F |
| VISTA (human) AlphaLISA Detection Kit | 500 assay points | AL3059C |
| | 5000 assay points | AL3059F |
| ½ AreaPlates (96-well, white) | 50 plates | 6005560 |
| | 200 plates | 6005569 |
| OptiPlate® (96-well, white) | 50 plates | 6005290 |
| | 200 plates | 6005299 |
| OptiPlate (384-well, white) | 50 plates | 6007290 |
| | 200 plates | 6007299 |
| AlphaPlate® (384-well, gray) | 50 plates | 6005350 |
| | 200 plates | 6005359 |
| AlphaPlate (1536-well, gray) | 50 plates | 6004350 |
| TopSeal®-A PLUS | Box of 100 | 6050185 |

**PerkinElmer, Inc.**
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
**www.perkinelmer.com**

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**For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs**



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