# Cytokine Detection In Culture Media Containing 10% FBS – A Comparison Of Two Commonly Used Homogeneous High Throughput Assay Technologies

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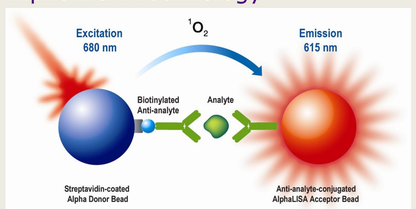
## 1 Abstract

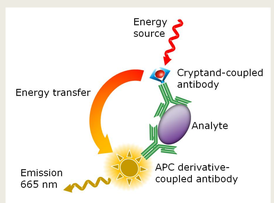
The aim of this work was to compare the performance of AlphaLISA® amplified luminescent proximity homogeneous assay technology platform and an alternative time-resolved fluorescence resonance energy transfer (TR-FRET) technology assay platform for the detection of cytokines in high-thoughput screening. Many cytokines are important biomarkers for inflammation. As they are often studied using cell-based assays, components in the sample matrix could affect the performance of homogeneous assays. A typical assay may contain culture medium with fetal bovine serum (FBS), from 1% to 10%. In this study, the cytokines IL1 and IL6 were assayed with the two technologies in the presence of DMEM-F12 culture media plus 10% heat-inactivated FBS. Critical parameters for an effective high-throughput assay include the sensitivity, the reproducibility (%CV), and the dynamic range. The time needed to collect data and analyze results is another important factor. Assay platforms that can deliver reliable results in a shorter period of time are highly desirable. AlphaLISA assays were supplied by PerkinElmer, and the alternative TR-FRET technology based kit was obtained from an alternative supplier. All assays were read on an EnVision® Multilabel detection instrument (PerkinElmer, Inc.). In the present study, the AlphaLISA assays exhibited better sensitivity, precision, and a larger dynamic range than the alternative TR-FRET technology assay for the two analytes tested. The AlphaLISA assay format also required 50% less biological sample volume, shorter incubation times, and simpler data manipulation. We also present data using a one-step, streamlined AlphaLISA assay protocol that can simplify workflows while maintaining high sensitivity.

## 2 Introduction

### AlphaLISA Technology




### Alternative TR-FRET Technology

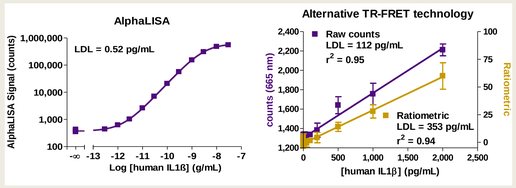


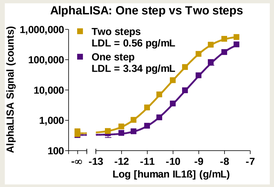

### TR-FRET calculations

TR-FRET data was processed in two ways, as "raw data" using the unaltered 665 nm counts (only the output signal from the FRET acceptor dye) and as a ratiometric analysis done according to the instructions provided in suppliers package insert.

## 3 Interleukin 1 beta

Sensivitiy:
Lower detection limit (LDL = mean of background value + 3SD)




Full reported linear range for alternative TR-FRET technology (10 ng/ml) was not tested because of the limited supply of the calibrator.

AlphaLISA assay is **more sensitive** than the alternative TR-FRET technology with both the two-step and one-step protocols.

Linear dynamic range:

| Range | AlphaLISA (Two-step protocol) | TR-FRET (Raw) | TR-FRET (Ratiometric) |
|---|---|---|---|
| Minimum | 0.52 pg/mL | 112 pg/mL | 350 pg/mL |
| Maximum | 3 ng/mL | 10 ng/mL | 10 ng/mL |
| Total | 3.8 Log | 1.9 Log | 1.4 Log |

AlphaLISA has a **larger range**, though alternative TR-FRET technology has a linear range that is shifted slightly higher.

Recovery:
AlphaLISA spike-in concentrations: 20, 100 and 1000 pg/mL.
TR-FRET spike-in concentrations: 150, 500 and 1000 pg/mL.
Unit NIBSC/WHO standard equivalency:
PerkinElmer = 11 pg, Alternative supplier = 10 pg

| Spiking | Concentrations | AlphaLISA | TR-FRET (Raw) | TR-FRET (Ratiometric) |
|---|---|---|---|---|
| % recovery spike-in | High | 97.8 | 103.6 | 111.2 |
| | Medium | 87.3 | 103.8 | 93.8 |
| | Low | 110.8 | 93.8 | 186.8 |
| % recovery alternative supplier spike-in | High | 104.8 | 85.9 | 0.84 |
| | Medium | 88.3 | 87.8 | -13.8 |
| | Low | 94.3 | 54.1 | -128.5 |

Gold standards should be utilized for unbiased determinations; standards from different vendors demonstrate substantial differences when cross-tested with competitive technologies.

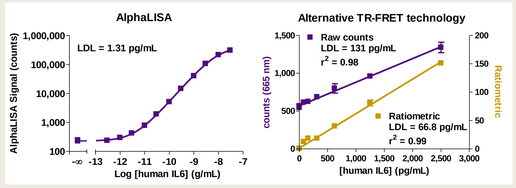
Precision:
Intra-assay: 9 replicates, Inter-assay: 3 x 9 replicates

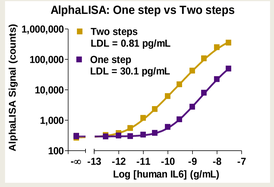
| % CV | Concentrations | AlphaLISA | TR-FRET (Raw) | TR-FRET (Ratiometric) |
|---|---|---|---|---|
| Intra-assay precision | High | 8.1 | 16.4 | 20.7 |
| | Medium | 4.4 | 12.8 | 24.9 |
| | Low | 5.6 | 33.1 | 33.4 |
| Inter-assay precision | High | 9.9 | 23.3 | 10.6 |
| | Medium | 11.6 | 61.5 | 37.0 |
| | Low | 11.9 | 76.4 | 78.7 |

AlphaLISA has much **better intra- and inter-assay precision** at all tested concentrations.

## 4 Interleukin 6

Sensivitiy:
Lower detection limit (LDL = mean of background value + 3SD)




Full reported linear range for alternative TR-FRET technology (12.5 ng/ml) was not tested because of the limited supply of the calibrator.

AlphaLISA assay is **more sensitive** than the alternative TR-FRET technology with both the two-step and one-step protocols.

Linear dynamic range:

| Range | AlphaLISA (Two-step protocol) | TR-FRET (Raw) | TR-FRET (Ratiometric) |
|---|---|---|---|
| Minimum | 1.3 pg/mL | 66.8 pg/mL | 131 pg/mL |
| Maximum | 7.5 ng/mL | 12.5 ng/mL | 12.5 ng/mL |
| Total | 3.8 Log | 2.3 Log | 2.0 Log |

AlphaLISA has a **larger range**, though the alternative TR-FRET technology has a linear range that is shifted slightly higher.

Recovery:
AlphaLISA spike-in concentrations: 50, 100 and 1000 pg/mL.
TR-FRET spike-in concentrations: 100, 500 and 1000 pg/mL.
Unit NIBSC/WHO standard equivalency:
PerkinElmer = 22 pg, Alternative supplier = 10 pg

| Spiking | Concentrations | AlphaLISA | TR-FRET (Raw) | TR-FRET (Ratiometric) |
|---|---|---|---|---|
| % recovery spike-in | High | 86.8 | 100.7 | 108.7 |
| | Medium | 101.6 | 100.9 | 105.8 |
| | Low | 96.0 | 82.7 | 100.4 |
| % recovery alternative supplier spike-in | High | 205.6 | 97.1 | 58.5 |
| | Medium | 152.4 | 82.0 | 55.0 |
| | Low | 134.7 | 46.1 | 349.7 |

Gold standards should be utilized for unbiased determinations; standards from different vendors demonstrate substantial differences when cross-tested with competitive technologies.

Precision:
Intra-assay: 9 replicates, Inter-assay: 3 x 9 replicates

| % CV | Concentrations | AlphaLISA | TR-FRET (Raw) | TR-FRET (Ratiometric) |
|---|---|---|---|---|
| Intra-assay precision | High | 9.4 | 23.0 | 14.1 |
| | Medium | 8.1 | 24.7 | 33.5 |
| | Low | 8.1 | 142.0 | 61.7 |
| Inter-assay precision | High | 14.9 | 17.3 | 17.9 |
| | Medium | 11.6 | 38.3 | 25.5 |
| | Low | 15.2 | 193.4 | 127.0 |

AlphaLISA has much **better intra- and inter-assay precision** at all tested concentrations.

## 5 Materials & Methods

Plates and equipement
AlphaLISA: OptiPlate-384 white (PerkinElmer, #6007299)
TR-FRET: 384 low-volume black (Greiner, #784076)
EnVision Multilabel Plate Reader

Matrix components (DMEM F12 + 10% Hi FBS)
DMEM F12 (Invitrogen, #11039-021)
Heat-inactivated FBS (Wisent, #080450)

Kits
IL1β AlphaLISA kit (AL220C)
IL6 AlphaLISA kit (AL223C)

### AlphaLISA Protocol (two-step)

1. 5 µL analyte standard
2. 20 µL mixture of anti-analyte Acceptor beads and biotinylated anti-analyte
3. Incubation at room temperature for 30 minutes
4. 25 µL streptavidin Donor beads
5. Incubation at room temperature in the dark for 60 minutes
6. Read on EnVision

### AlphaLISA Protocol (one-step)

1. 5 µL analyte standard
2. 20 µL mixture of anti-analyte Acceptor beads and biotinylated anti-analyte
3. 25 µL streptavidin Donor beads
4. Incubation at room temperature in the dark for 90 minutes
5. Read on EnVision

### TR-FRET Protocol

1. 10 µL analyte standard
2. 5 µL of anti-analyte Ab-cryptand
3. 5 µL of anti Ab-APC derivative (acceptor)
4. Incubation at room temperature for 2-3 hours
5. Read on EnVision

## 6 Summary

In comparison to an alternative TR-FRET technology in the detection of cytokines in a complex matrix, the AlphaLISA kits were shown to have:

- Greater window (linear dynamic range),
- Lower detection limit (even with a one-step protocol), and
- Higher intra- and inter-assay precision (lower %CV)

The advantages of using AlphaLISA over an alternative TR-FRET technology are:

- Lower sample volume requirement yet better performance
- Compatibility with both serum samples and cell supernatants
- Shorter total assay duration
- Simpler data analysis

This comparative study demonstrates the markedly enhanced performance and ease of use of the AlphaLISA technology over that of an alternative homogeneous TR-FRET technology platform.