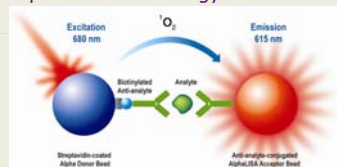


1 Abstract

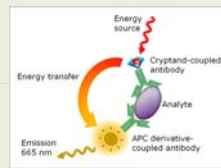
The aim of this work was to compare the performance of AlphaLISA® amplified luminescent proximity homogeneous assay technology platform and an alternative time-resolved fluorescence resonance energy transfer (TR-FRET) technology assay platform for the detection of cytokines in high-throughput screening. 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

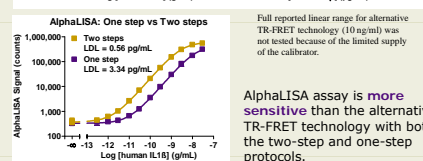
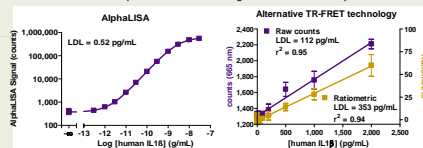


TR-FRET calculations
TR-FRET data was processed in two ways, as "raw data" using the unaltered 665 nm counts (only the output signal from the FRET acceptor dye) and as a ratiometric analysis done according to the instructions provided in suppliers package insert.

3 Interleukin 1 beta

Sensitivity:

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	353 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

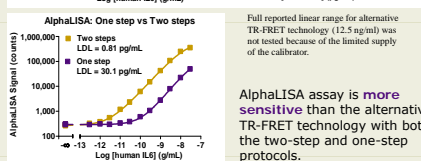
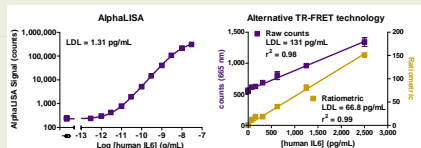
% CV	Concentrations	AlphaLISA	TR-FRET (Raw)	TR-FRET (Ratiometric)
Intra-assay precision	High	8.1	16.4	20.7
	Medium	4.4	12.8	24.9
	Low	5.6	33.1	33.4
Inter-assay precision	High	9.9	23.3	10.6
	Medium	11.6	61.5	37.0
	Low	11.9	76.4	78.7

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

4 Interleukin 6

Sensitivity:

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 equipment

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)

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

AlphaLISA Protocol (one-step)

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

TR-FRET Protocol

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