Excellent assay performance of THUNDER™ TR-FRET cell-based cytokine assays performed on the PHERAstar® *FSX*

Geneviève Chatel, Mireille Caron, and Jaime Padros
BioAuxilium Research, Inc., Montreal, Canada

- **Five THUNDER™ TR-FRET cytokine assays were tested for compatibility on the PHERAstar *FSX***
- **PHERAstar *FSX* exhibits much higher sensitivity and dynamic range than a competing HTS microplate reader equipped for TRF laser excitation**
- **THUNDER™ combined with PHERAstar *FSX* provides a powerful approach for cytokine quantification**

Introduction

Cytokines are important regulators of cell proliferation, differentiation, and immune response[1]. Since their roles are not completely understood yet, both basic and drug discovery research require accurate cytokine quantification methods. THUNDER™ Biomarker Assays are designed to enable the simple, rapid, sensitive, and robust quantification of biomarkers in cell supernatants. BioAuxilium's enhanced Time-Resolved Förster Resonance Energy Transfer (TR-FRET) technology[2] is well-suited for cytokine quantification, with distinct advantages over conventional ELISA: homogeneous format, low sample volume, one-step protocol, wider dynamic range, high-throughput capability, and proven robustness and reproducibility.

Here we describe the validation of the PHERAstar *FSX* multi-mode microplate reader for measuring a panel of five THUNDER™ human cytokine assays.

Assay Principle

THUNDER™ cytokine assays are homogeneous sandwich immunoassays (Figure 1). One antibody is labeled with a long lifetime Europium chelate donor (Eu-Ab1) and the second with a far-red acceptor fluorophore (FR-Ab2). Upon excitation of the Europium chelate at 320 or 340 nm, energy is transferred from the donor to the acceptor fluorophore if they are sufficiently close for FRET. The emission by the acceptor of a long-lived TR-FRET signal at 665 nm is measured after a time delay.

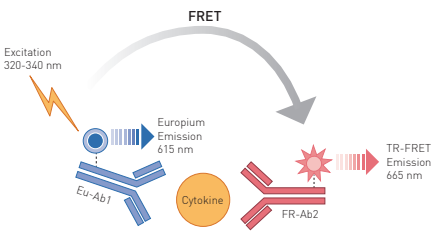

Fig. 1: THUNDER™ TR-FRET cytokine assay principle.

Materials & Methods

- 384-well, white low-volume plates (PerkinElmer®)
- KIT-IFNGP-100, KIT-IL1B-100, KIT-IL12-100, KITCCL2 100, and KIT-TNFA-100 assay kits (BioAuxilium Research)
- Human recombinant proteins (R&D Systems®)
- PHERAstar® *FSX* microplate reader (BMG LABTECH)

Experimental Procedure

Protocols were conducted as per BioAuxilium's recommendations[3]. The standard curves were run using each kit's Assay Buffer. Standards (15 µL) were added to 384-well white assay plates followed by the addition of the Antibody Mix (5 µL) for detection of the target cytokine. The plates were incubated at room temperature for the appropriate time and read on the PHERAstar *FSX* using the settings recommended for THUNDER™. For comparison, plates were also read on a competing HTS microplate reader equipped for TRF laser excitation. Data were expressed as (665/620) * 1,000 and are the mean ± SD of three wells per point. Data were fit to a 4PL model with 1/Y2 data weighting (GraphPad®). As per BioAuxilium's protocol, the limit of detection (LOD) and lowest limit of quantification (LLOQ) were calculated by adding 2 or 10 standard deviations (SD), respectively, to the mean background counts (zero standard; 12 replicate wells).

Instrument Settings

Optic settings	Time-resolved fluorescence, plate mode endpoint	
	Optic module	337 665 620
Integration time	Delay: 40 µsec, Time: 400 µsec	
General settings	Number of flashes	40
	Settling time	0.1 sec

Results & Discussion

Figure 2 shows a typical non-linear standard curve for human IL-1β. The assay read on the PHERAstar *FSX* showed an excellent well-to-well precision, with the mean percentage coefficient of variation (CV%) for the calibration point signals at 1.7% (3.8% for the competing HTS plate reader). The LOD and LOQ values were approximately 25-fold lower compared to those obtained with the competitor, regardless of the instrument settings used, thereby significantly extending the assay dynamic range. Furthermore, the signal-to-background (S/B) ratio at the highest standard was also higher with the PHERAstar *FSX*.

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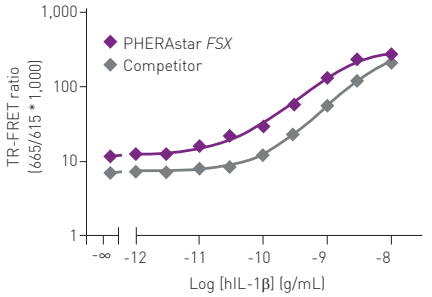

Fig. 2: THUNDER™ Human IL-1β standard curve.

The higher assay sensitivity of the PHERAstar *FSX* is evident in Figure 3, which shows a linear plot of the low-concentration part of the human IL-1β standard curve. For the PHERAstar *FSX*, there is a clear systematic signal increase over the 0-30 pg/mL range. This, combined with the very low CVs (1.9% versus 3.4% for the competing HTS plate reader), makes it possible to discriminate between these very low concentrations.

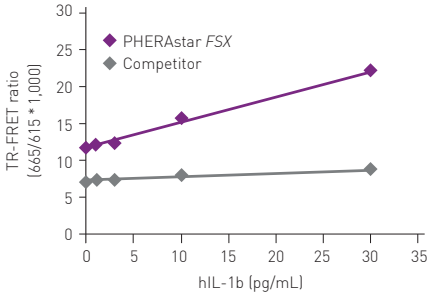

Fig. 3: A linear plot of the low concentration part of the THUNDER™ Human IL-1β standard curve.

The analytical (LOD) and functional (LLOQ) sensitivities obtained with the evaluated human cytokine assays are summarized in Table 1. In all cases, the PHERAstar *FSX* exhibited a higher assay sensitivity compared to the competitor and our internal data[3], decreasing the LOD to single digit pg/mL. These data demonstrate that the PHERAstar *FSX* is particularly advantageous for performing quantitative THUNDER™ cytokine assays.

Analyte	Assay parameter	PHERAstar *FSX*	Competitor
IFNγ	LOD (pg/mL)	6	24
	LLOQ (pg/mL)	33	121
	S/B	15	9
IL-1β	LOD (pg/mL)	1	26
	LLOQ (pg/mL)	7	141
	S/B	23	17
IL-12	LOD (pg/mL)	3	20
	LLOQ (pg/mL)	19	98
	S/B	22	15
TNFα	LOD (pg/mL)	3	13
	LLOQ (pg/mL)	41	62
	S/B	57	45
CCL2	LOD (pg/mL)	3	13
	LLOQ (pg/mL)	17	61
	S/B	17	15

Table 1: Performance of THUNDER™ cytokine assays on the PHERAstar *FSX* and the competing HTS reader.

Of note, systematic variations to the instrument settings (i.e., gain, delay, and integration time) used for reading the assays had no significant impact on the overall performance (LOD, LLOQ, S/B; data not shown), demonstrating the robustness of THUNDER™ cytokine assays. Readings of sample plates on eight different PHERAstar *FSX* were highly consistent and exhibited minimal variations (*e. g.*, standard deviation of 1.6% for slope of IL-1β standard curves).

Conclusion

The current data validate the compatibility of the PHERAstar *FSX* for THUNDER™ cytokine assays. All assays tested exhibited higher sensitivity and broader dynamic ranges compared to a competing HTS plate reader equipped for TRF laser excitation. The combination of simple, rapid, and robust yet affordable THUNDER™ cytokine assays with the highly sensitive PHERAstar *FSX* provides a powerful approach for accelerating the workflow of drug discovery and life science research.

References

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