Figure 3 summarizes results obtained with the Phospho-p38\(\alpha\beta\gamma\) (T180/Y182) assay kit. Incubation of HeLa cells (50,000 cells/well) with Anisomycin for 60 min at 37°C stimulates phosphorylation of p38\(\alpha\beta\gamma\) at T180/Y182. The assay shows a very high S/B ratio and the expected EC\(_{50}\)\(^5\).

Results obtained with the Phospho-STAT3 (Y705) assay kit are summarized in Figure 4. HeLa cells (40,000 cells/well) treated with Interferon-alpha (IFN\(\alpha\)\(^\_2\)) for 20 min at RT show the anticipated concentration-dependent activation of phospho-STAT3 at Y705, with a high S/B ratio and the expected EC\(_{50}\)\(^6\).

Overall, data were similar to those obtained with other TR-FRET enabled readers, demonstrating that the CLARIOstar\(\text{Plus}\) is suitable for performing THUNDER™ cellular assays. In addition, results obtained using different instrument settings [gain, delay and integration time] recommended for other TR-FRET technologies had no significant impact on the overall THUNDER™ assay performance [data not shown], further highlighting the robustness of THUNDER™ cellular kinase assays.

Conclusion

The current data validate the compatibility of the CLARIOstar\(\text{Plus}\) for THUNDER™ cellular kinase assays. All assays exhibited high signal levels and S/B ratios, broad dynamic ranges, and the expected potency. THUNDER™ cellular assays run on the CLARIOstar\(\text{Plus}\) are well-suitable for both basic research and drug discovery applications and offer a straightforward approach for the determination of protein phosphorylation levels in cell lysates.

References


Fig. 2: THUNDER™ Phospho-ERK1/2 (T202/Y204) assay (4 hours incubation).

Fig. 3: THUNDER™ Phospho-p38\(\alpha\beta\gamma\) (T180/Y182) assay (overnight incubation).

Fig. 4: THUNDER™ Phospho-STAT3 (Y705) assay (overnight incubation).
Excellent assay performance of THUNDER™ TR-FRET cytokine assays performed on the PHERAstar® FSX

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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. 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 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.

Materials & Methods

- 384-well, white low-volume plates (PerkinElmer®)
- KIT-IFNGP-100, KIT-IL1B-100, KIT-IL12-100, KITCCL2100, 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. 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

<table>
<thead>
<tr>
<th>Optic settings</th>
<th>Time-resolved fluorescence, plate mode endpoint</th>
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<tbody>
<tr>
<td>Optic module</td>
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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.