Validation of a Suite of Invitrogen Assay Technologies on BioTek® Instruments

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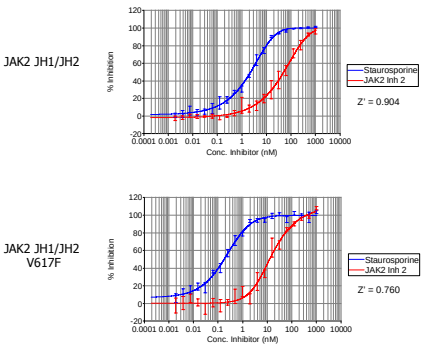
Introduction

Robust, sensitive assays suitable for high-throughput applications are critical for drug discovery. It is also critical to pair these assays with a suitable instrument; ideally, a single plate reader flexible enough to be capable of reading multiple technologies and suitable for use in evaluation labs as well as downstream HTS and lead discovery groups.

Invitrogen is proud to offer a suite of assay formats for HTS and compound profiling. We offer assays based upon a number of fluorescent technologies, including fluorescence intensity, FRET, and TR-FRET to provide tools for screening kinases, nuclear receptors, and other biologically relevant proteins. In this poster, we demonstrate the utility of two BioTek instruments, the Synergy™ 2 Multi-Detection Microplate Reader and the FLx800™ Multi-Detection Microplate Reader. We evaluated multiple Invitrogen assay formats including those for kinetic, cell-based, biochemical and drug metabolism. The BioTek Synergy™ 2 Reader has been successfully validated with Invitrogen's GeneBLAzer®, Z'-LYTE®, LanthaScreen™, Omnia®, and Vivid® assay technologies. We demonstrate that the BioTek FLx800™ Reader is also suitable for all technologies tested except LanthaScreen™ assays. Both instruments offer multiple options for drug discovery screening in cellular and biochemical formats, as well as analysis of P450-mediated compound metabolism.

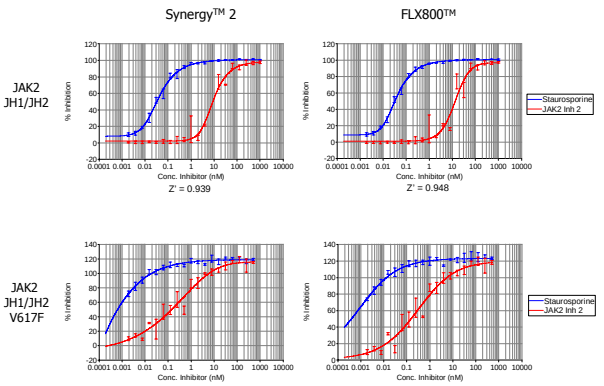
Synergy™ 2 FLx800™

Figure 1 – LanthaScreen™ TR-FRET Assays on the Synergy™ 2



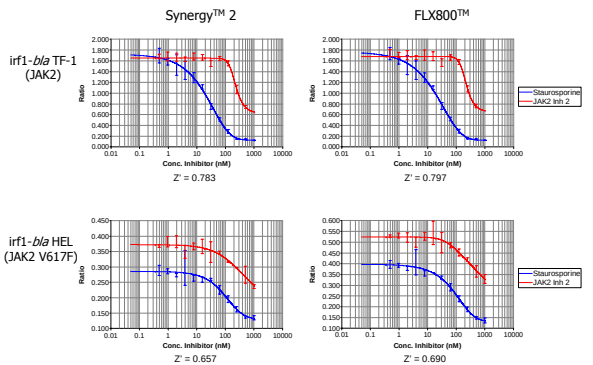
LanthaScreen™ assay performance was assessed using JAK2 JH1/JH2 (PV4393, 55.5 ng/ml) and JAK2 JH1/JH2 V617F (PV4336, 77 ng/ml) and 400 nM fluorescein-GT (Cat. no. PV3610) as substrate in the presence of a titration of staurosporine and JAK2 Inhibitor II. After a one-hour reaction, EDTA and Tb-labeled PY20 antibody (Cat. no. 3552) were added to final concentrations of 10 mM and 1 nM, respectively. The final volume for assay readout was 20 µl, and the assay was run in a black Corning low-volume 384-well plates. Data analysis was performed using BioTek Gen5® software. Z' factors were determined from 6 negative controls (no kinase) and 6 positive controls (fully active kinase) per plate.

Figure 2 – Z'-LYTE® FRET-Based Assays on the Synergy™ 2 and FLx800™



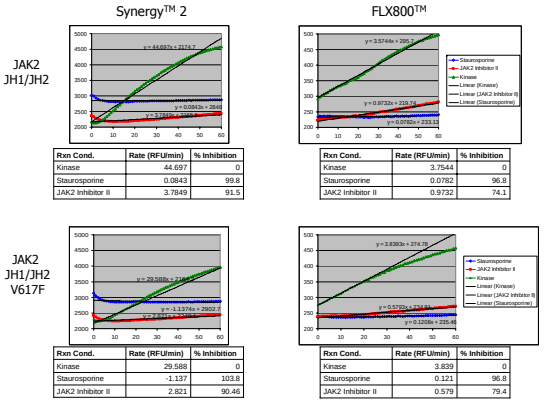
Z'-LYTE® assay performance was assessed using JAK2 JH1/JH2 (PV4393, 648.6 ng/ml) and JAK2 JH1/JH2 V617F (PV4336, 326.6 ng/ml) and the Z'-LYTE® Tyr 6 kit (Cat. no. PV4122). The final 10 µL Kinase Reaction consists of JAK2 kinase and 2 µM Tyr 06 Peptide in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl2, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of Development Reagent A is added, at the lot-specific dilution indicated. The final volume for assay readout was 20 µl, and the assay was run in a black Corning low-volume 384-well plates. Data analysis was performed using BioTek Gen5® software. Z' factors were determined from 6 negative controls (no kinase) and 6 positive controls (fully active kinase) per plate.

Figure 3 – GeneBLAzer® Cell-Based Assays on the Synergy™ 2 and FLx800™



GeneBLAzer® assay performance was assessed using the CellSensor® Irf1-*bla* TF-1 cell line (K1219, expressing JAK2 when induced by GM-CSF stimulation) and the CellSensor® irf1-*bla* HEL cell line (K1238, a human erythroleukemia line with a homozygous JAK2 V617F mutation). Assays were performed as directed in the protocols for each line, using a titration of staurosporine and JAK2 Inhibitor II. The final volume for assay readout was 120 µl, and the assays were run in black-wall, clear-bottom 96-well assay plates from Corning. Plates were read from the bottom and data analysis was performed using BioTek Gen5® software. Z' factors were determined from 6 negative controls of unstimulated cells and 6 positive controls of GM-CSF-stimulated cells for the TF-1 cell line, and from 6 positive controls of untreated cells and 6 negative control wells treated with a maximal dose of staurosporine for the HEL line per plate.

Figure 4 – Omnia® Kinetic Assays on the Synergy™ 2 and the FLx800™



Rxn Cond.	Rate (RFU/min)	% Inhibition
Kinase	44.697	0
Staurosporine	0.0843	99.8
JAK2 Inhibitor II	3.7849	91.5

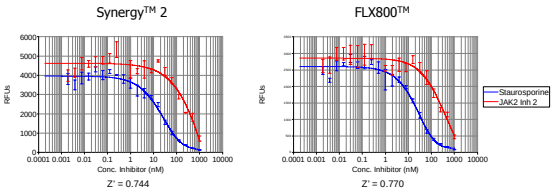
Rxn Cond.	Rate (RFU/min)	% Inhibition
Kinase	3.7544	0
Staurosporine	0.0782	96.8
JAK2 Inhibitor II	0.9732	74.1

Rxn Cond.	Rate (RFU/min)	% Inhibition
Kinase	29.588	0
Staurosporine	-1.137	103.8
JAK2 Inhibitor II	2.821	90.46

Rxn Cond.	Rate (RFU/min)	% Inhibition
Kinase	3.839	0
Staurosporine	0.121	96.8
JAK2 Inhibitor II	0.579	79.4

Omnia® assay performance was assessed using JAK2 JH1/JH2 (PV4393, 5 µg/ml) and JAK2 JH1/JH2 V617F (PV4336, 5 µg/ml) and 10 µM of an unreleased Tyrosine kinase substrate in the presence of 1 µM staurosporine and JAK2 Inhibitor II. The final volume for assay readout was 50 µl, and the assay was run for 60 minutes taking readings at 1-minute intervals, using the white 96-well low-volume plates provided with Omnia assay kits. Results are plotted as a 60 minute timecourse, using single wells of kinase alone, kinase + staurosporine and kinase + JAK2 Inhibitor2. Data was plotted using Microsoft Excel.

Figure 5 – Vivid® CYP450 Assays on the Synergy™ 2 and the FLx800™



Vivid® assay performance was assessed using the Vivid® CYP3A4 Blue (P2858) kit in the presence of a titration of staurosporine and JAK2 Inhibitor II. The final reaction volume was 20 µl, with 10 µM BOMCC, 5 nM Baculosomes® CYP3A4, and 250 µM NADP+, and the assay was run for 60 minutes before stopping the reaction with 2 µl of 100 µM ketoconazole. Assay was performed in black low-volume non-treated Costar plates. Data analysis was performed using BioTek Gen5® software. Z' factors were determined from 6 negative controls (no P450 added) and 6 positive controls (no inhibitors added) per plate.

Conclusions

- The BioTek Synergy™ 2 and FLx800™ are suitable for a broad range of Invitrogen's fluorescent assay technologies.
- Both instruments are capable of bottom-reading, allowing use of LiveBLAzer® loading substrates for GeneBLAzer® assays. Furthermore, the Synergy™ 2 is also capable of performing TR-FRET assays, making it suitable for LanthaScreen™ kinase, ubiquitination, and nuclear receptor assays.
- Both instruments are capable of highly robust, reproducible results using most Invitrogen assays, allowing compound screening in biochemical and cell-based formats, as well as CYP450-inhibition screening, in a single, sensitive and low-cost instrument.
- Full setup documents detailing setup of the Synergy™ 2, Synergy™ 4, and FLx800™ for Invitrogen screening assays will be available soon based upon the data included here.

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