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Application Note

GeneBLAzer® FRET Cell-based Assay from Invitrogen™ on Synergy™ 4

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Introduction

Here we describe the use of the Synergy 4 Hybrid Microplate Reader to measure the output from Invitrogen's GeneBLAzer® FRET cell-based assay technology used to study various drug target classes including G protein-coupled receptors, nuclear hormone receptors, and signaling pathways implicating various protein kinases. GeneBLAzer® technology uses transformed cell lines to express a beta-lactamase reporter gene that is controlled by regulatory elements responsive to the cellular pathway of interest.

The basis for detection of the GeneBLAzer® assay is the membrane-permeant FRET based substrate. This noninvasive technique uses a CCF4 substrate where the carboxylate groups have been derivatized as esters resulting in a nonpolar compound (CCF4-AM) that is permeable to cell membranes. Once inside the cell, the compound is hydrolyzed by intracellular esterases (Figure 1). The resultant activated indicator is now a polar molecule that is no longer capable of freely diffusing through the cell membrane, essentially trapping the compound inside the cell. The CCF4 molecule has two fluorescent moieties, coumarin and fluorescein linked together with a beta-lactam ring. In the absence of beta-lactamase activity the unreacted substrate molecule remains intact. Excitation of the coumarin by 409 nm light results in fluorescence resonant energy transfer (FRET) to the fluorescein moiety, which can be detected by green 520 nm fluorescence. In the presence of beta-lactamase expression, the CCF4 substrate is cleaved at the beta-lactam ring and the FRET is disrupted. Under these conditions, excitation of the coumarin results in the emission of blue 447 nm fluorescence (Figure 1). Beta-lactamase expression is quantified by measuring the ratio of the blue product (447 nm) to the green substrate (520 nm) fluorescence.

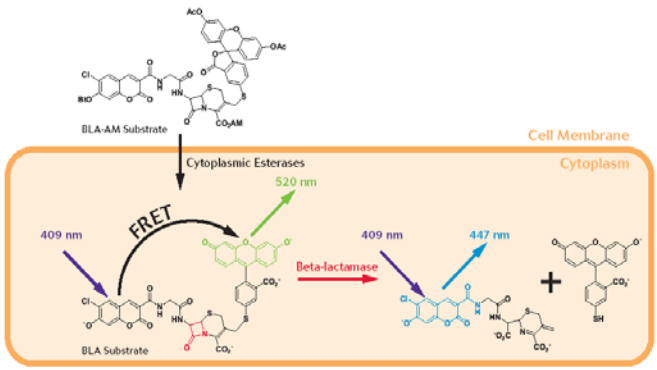

Figure 1. Schematic illustration of GeneBLAzer® assay. The CCF4 substrate has coumarin and fluorescein moieties linked together via a beta-lactam ring. Unreacted substrate is capable of FRET upon excitation with 409-nm light, whereas reacted products are not.

Materials and Methods

CellSensor® irf1-*bla* TF1 cell line (catalog number K1219), Phosphate Buffered Saline without calcium and Magnesium [PBS(-)] (catalog number 14190-136), a LiveBLAzer™ FRET Loading Kit (catalog number K1095) and Solution D (catalog number K1156) were supplied by Invitrogen (Carlsbad, CA). Black, clear bottom 96-well (catalog number 3603) and 384-well (catalog number 3712) plates were obtained from Corning.

The irf1-*bla* TF1 cells were seeded at a density of 5 x 10^5 cells/ mL into 96-well plates in a volume of 100 µL (50,000 cells) and in 40 µL (20,000 cells) in 384-well plates. The following morning, a generic kinase inhibitor, staurosporine, was added to the cells at the indicated concentrations. The cells were then stimulated with GM-CSF and allowed to incubate 4-5 hours at 37°C with 5% CO_2. After treatment, cells in 96-well and 384-well microplates were loaded with 20 µL and 8 µL of 6X loading solution, respectively, and allowed to incubate in the dark at room temperature for 2-2.5 hours.

Fluorescent measurements were made using a Synergy 4 Hybrid Microplate Reader. All measurements were made from the bottom using a 400/30-excitation filter and both a 460/40 and 528/20 emission filters.

Results

The data presented in Figure 2 demonstrates that the Synergy 4 Hybrid Microplate Reader is capable of detecting the inhibition of beta-lactamase expression by staurosporine. Beta-Lactamase activity hydrolyses the CCF4 substrate into two separate moieties, which in turn eliminates FRET from occurring within the molecule. A low level of beta-lactamase activity is indicated by a low 460/528 ratio. With increasing concentrations of staurosporine the 460/528-emission ratio decreases, indicating that there is less beta-lactamase activity present.

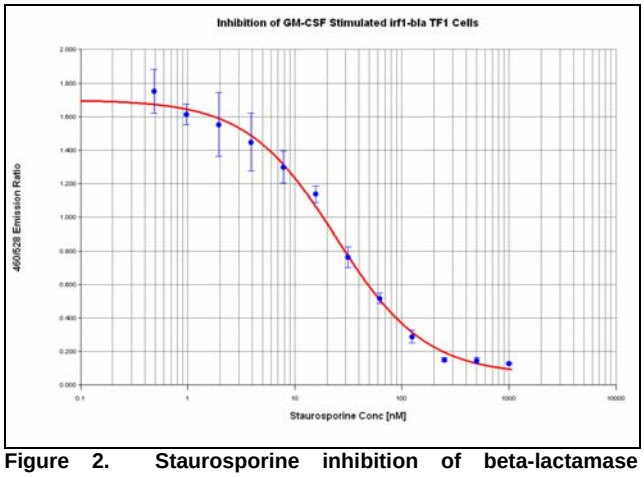

Figure 2. Staurosporine inhibition of beta-lactamase expression in irf1-*bla* TF1 cells cultured in 96-well microplates.

Cells cultured in 384-well plates can also be measured for beta-lactamase activity under conditions of increasing staurosporine concentrations. As demonstrated in Figure 3, staurosporine effectively inhibits the expression of beta-lactamase in GM-CSF stimulated irf1-*bla* TF-1 cells plated in 384-well microplates.

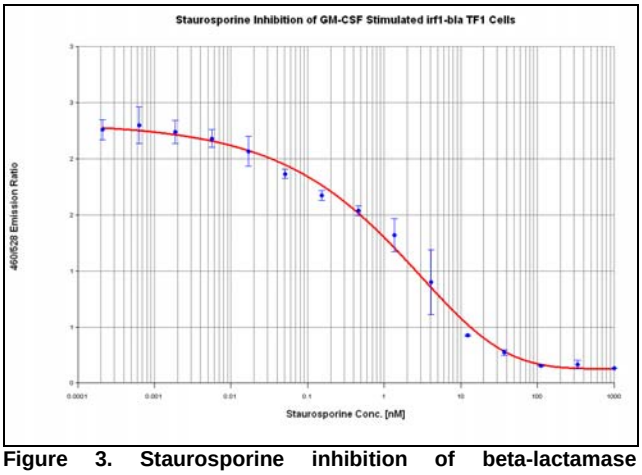

Figure 3. Staurosporine inhibition of beta-lactamase expression in irf1-*bla* TF1 cells cultured in 384-well microplates.

Acknowledgements

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