Cellular and Biochemical Assay Applications on the Varioskan™ LUX multimode microplate Reader

Chetana Revankar , Justin Wetter, Jacquelyn Webb, Kyle Kimler, Robert Horton and David Piper
Cell Biology, Research and Development, Thermo Fisher Scientific, 5781 Van Allen Way, Carlsbad, CA 92008 USA

invitrogen by Thermo Fisher Scientific

Overview

High-throughput assay development for cellular or biochemical profiling of drug candidates requires the use of multiple assay formats that necessitate versatility in plate reader detection formats. The ability to use a single plate reader platform across multiple assay formats eliminates the need, footprint and expense of having multiple instruments in your laboratory. Here we demonstrate the use of a wide array of Thermo Fisher Scientific cellular and biochemical assays for cellular pathway analysis, including cell health, and targeted kinase and GPCR assay development on the Varioskan LUX. This instrument platform provides high sensitivity, accuracy and precision when measuring multiple detection modalities: chemiluminescence, fluorescence (Forster) resonance energy transfer (FRET) and time-resolved FRET (TR-FRET), as illustrated by its performance in assessing cell health using our PrestoBlue™ reagent, kinase activity using our LanthaScreen™ TR-FRET platform, protein-protein interactions using our Split luciferase technology and pathway readouts that rely on either our Turbo luciferase system or our CellSensor™ pathway assays. This work demonstrates how a multimode microplate reader like Varioskan™ LUX from Thermo Fisher Scientific in conjunction with Thermo Fisher Scientific cellular and biochemical assay technologies form a powerful combination for versatile assay development for high-throughput drug discovery.

Varioskan™ LUX multimode microplate reader

The Thermo Scientific Varioskan LUX combines simplicity with top-end performance. Designed for researchers with a wide variety of needs, Varioskan LUX offers features to save time and help reduce common errors in the lab - including automated dynamic range selection, which adjusts the optimal reading range based on signal intensities, and built-in safety controls, which alert you to potential errors before they begin.

Ideal for applications including absorbance (UV-Vis), fluorescence intensity, luminescence, and time-resolved fluorescence.

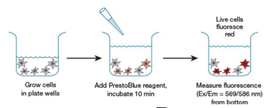
Plate types for various applications include:
Absorbance: 6-384 well plates
Fluorescence: 6-1536 well plates
Time-Resolved Fluorescence: 6-1536 well plates
Luminescence: 6-1536 well plates (spectral scanning 6-384 well plates)

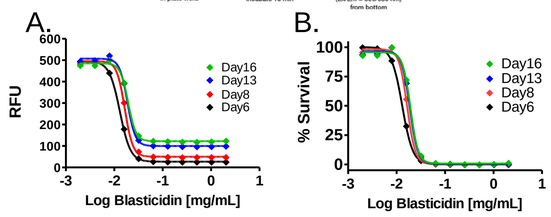
The newly designed 4th generation SkanIt software is easy-to-navigate and the interface will guide you through the measurement process and getting the results you need. SkanIt is available in two editions: Research Edition for scientists working in life science research, and Drug Discovery Edition that provides features to help you comply with the requirements of FDA 21 CFR Part 11.

Cell Health / Prestoblue™ Assay

Rapid quantification of cell viability

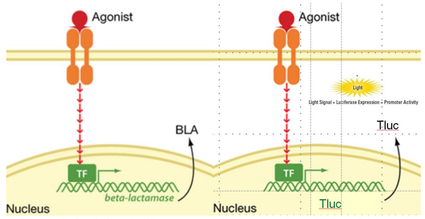
- Save time with a shorter incubation step than other resazurin-based assays
- Obtain accurate results with proven consistency
- Monitor living cells over time
- Absence of cell lysis step enables downstream or multiplexed functional



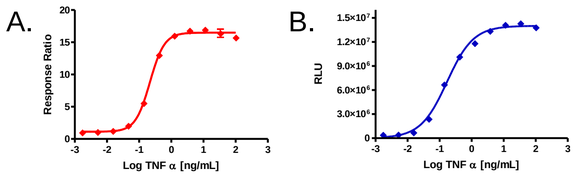


Kill Curve for Blasticidin Sensitivity: U2OS cells were plated in a 96-well plate at 10,000 cells per well and then exposed to various concentrations of blasticidin for a period of 16 days. On day 6, PrestoBlue™ reagent was added to the cells (1X final conc.). The cells were incubated at 37ºC for 2 hours prior to reading the fluorescence on Varioskan LUX. **A.** Data was plotted as Relative Fluorescence Values (RFU) versus the log. concentration of blasticidin. **B.** Data from plot A was normalized for baseline correction and plotted as percent survival versus the log. concentration of blasticidin. As determined from this assay, 65 µg of blasticidin per mL was the lowest concentration of blasticidin that resulted in 100% cell death.

CellSensor™ assays and Jump-In™ platform



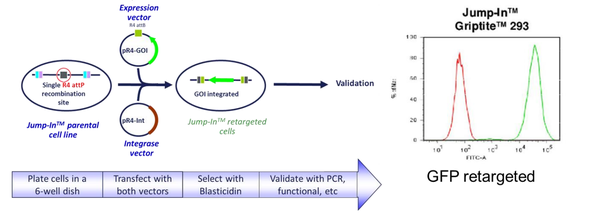
CellSensor Assay Principle: CellSensor cell lines use GeneBLAzer™ beta-lactamase (BLA) reporter technology or can be customized to express luciferase reporter to provide you with a rapid and sensitive method of analyzing signal transduction pathways exposed to drug candidates or other stimuli. These cell lines measure compound potency and selectivity and allow for analysis, in a high-throughput manner, of key pathways activated or down-regulated by compounds.

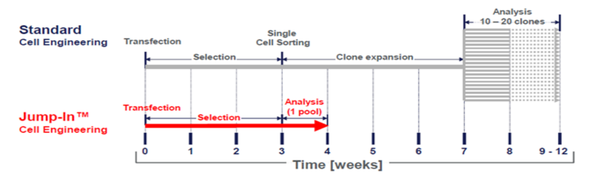


CellSensor Assay: The cells were plated in 384-well plates at 20,000 cells per well in growth medium. Next day the cells were stimulated with various concentrations of TNFα for 5 h. **A. CellSensor NF-κB-bla HEK293 cell line:** BLA activity was measured using GeneBLAzer assay kit. Briefly, cells were loaded with CCF4 for 2 h at RT. Emission data at 440 and 530 nm was collected using an excitation wavelength of 409 nm using Varioskan LUX. Data is plotted as response ratio (stimulated vs unstimulated sample) against the concentrations of TNFα **B**. **Jump-In NF-κB TurboLuc HEK293 cell line:** Luciferase activity was measured using TurboLuc™ Luciferase one-step glow assay kit. The growth medium was aspirated and 80 µL of 1X assay buffer with substrate was added to each well. Following which, the luciferase activity was recorded using Varioskan LUX. Data is plotted as Relative Luminescence Units (RLU) against the concentrations of TNFα

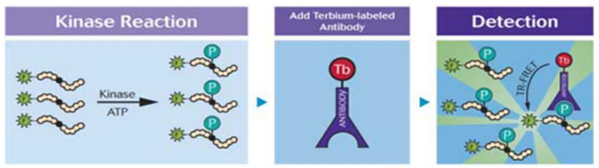
Jump-In cell line development

- **Rapid and efficient generation of engineered cell lines**
  - Functional cell pools can be generated in as little as 3 weeks
  - No laborious clone generation and analysis
  - Accepts large multi-gene inserts
- **Isogenic expression**
  - All cell lines derived from a given parental Jump-In™ cell line express the gene of interest from the same genomic locus.

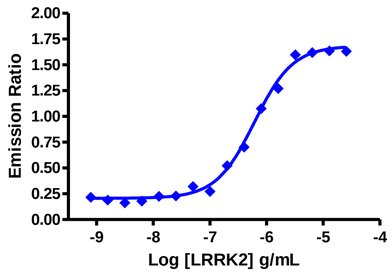




LanthaScreen™ Activity TR-FRET assay



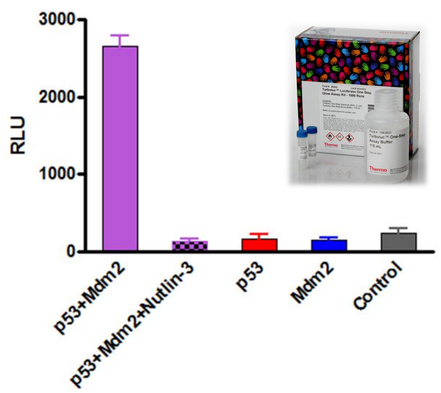
The LanthaScreen kinase activity assay: In a LanthaScreen kinase activity assay, the kinase, fluorescein-labeled substrate, and ATP are allowed to react. Then EDTA (to stop the reaction) and terbium-labeled antibody (to detect phosphorylated product) are added. In a LanthaScreen kinase reaction, the antibody associates with the phosphorylated fluorescein labeled substrate resulting in an increased TR-FRET value. The TR-FRET value is a dimensionless number that is calculated as the ratio of the acceptor (fluorescein) signal to the donor (terbium) signal. The amount of antibody that is bound to the tracer is directly proportional to the amount of phosphorylated substrate present, and in this manner, kinase activity can be detected and measured by an increase in the TR-FRET value.



Titration of LRRK2 WT protein: The indicated concentrations of LRRK2 were incubated at room temperature with 400 nM LRRKtide substrate and 500 nM ATP for 1 h. Tb-anti-pERM antibody and stop solution was then added and TR-FRET was recorded on Varioskan LUX using the TR-FRET module

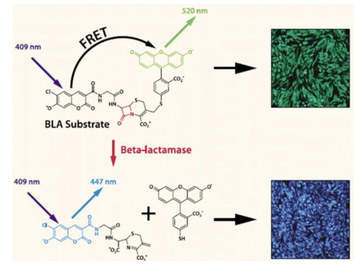
Protein-Protein interaction assay

PPI	Schematic	Pros	Cons
Split Luciferase	A B N-Luc C-Luc + substrate → A B Luc	• Highly sensitive - can detect interactions at endogenous levels •Can detect reversible interactions (does not trap complexes)	•Requires luciferase substrate



Split Turbo Luciferase assay: 293FT cells were plated in a 96-well plate at 40,000 cells per well in growth medium. Next day the cells were transfected with either pSTL-N1-p53 or pSTL-N2-Mdm2 or both plasmids. Twenty-four hours later the cells were also treated with Nutlin-3 to disrupt the p53 and Mdm2 interaction. The luciferase activity in the cells was measured using TurboLuc™ Luciferase one-step glow assay kit. The growth medium was aspirated and 100 µL of 1X assay buffer with substrate was added to each well. Following which, the luciferase activity was recorded using Varioskan LUX

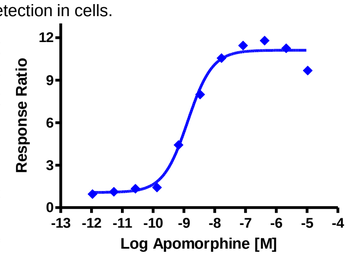
GeneBLAzer™ assay for GPCRs



Cells are loaded with CCF4 substrate containing two fluoroprobes, coumarin and fluorescein. In the absence of BLA expression, the substrate molecule remains intact. In this state, excitation of the coumarin results in FRET to the fluorescein moiety and emission of green light. However, in the presence of BLA expression, the substrate is cleaved, separating the fluorophores, and disrupting FRET. Excitation of the coumarin in the presence of BLA results in a blue fluorescence signal. The resulting blue:green ratio provides a normalized reporter response.

GeneBLAzer Technology uses bla gene combined with a FRET-enabled substrate to provide reliable and sensitive detection in cells.

GeneBLAzer D2-Gqo5-NFAT-bla CHO-K1 dose response under optimized conditions: The cells (10,000 cells/well) were plated in a 384-well format and incubated for 16-20 hours. Cells were stimulated with a dilution series of apomorphine for 5 hours. Cells were then loaded with CCF4 substrate for 2 h. Fluorescence emission values at 440 nm and 530 nm were measured and the response ratio was plotted against the concentrations of apomorphine.



ThermoFisher SCIENTIFIC

For Research Use Only. Not for use in diagnostic procedures.