GloMax® Discover – Ideal Partnership of Assays and Reagents

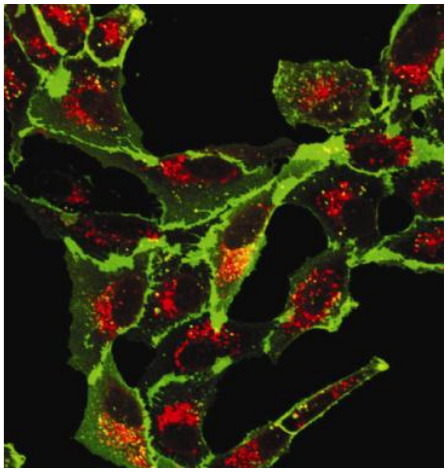
Craig Malcolm: Product Manager Cell Analysis & Proteomics

Cell-Based Assay Tour - March2014

Outline

- Overview of GloMax® Discover
- Assay Performance
- Automation Integration

Promega capabilities



Cellular & Biochemical Technologies

- Assay Design
- Integrated Cellular Biology
- Macromolecular Design
- Protein Analysis
- Organic Chemistry

Nucleic Acid Technologies

- Purification
- Amplification
- Detection

Instrument & Reagent Technologies

- Instrumentation
- Reagents
- Software
- Services

Introducing …

- Integrated with Promega assays
- Multi-mode detection (Luminescence, Fluorescence, UV-Vis Absorbance, BRET, FRET, filtered luminescence)
- Easy to use: Plug-n-play setup
- Superior sensitivity, dynamic range, and cross-talk performance
- Integrated filter paddles for assay multiplexing
- 6 to 384-well plate formats
- Automation-friendly
- Tablet PC touchscreen control and wireless connectivity to network and Promega.com
- Electronic signature control for 21CFR Part 11
- IQ / OQ Service

GloMax
DISCOVER

Integrated with Promega Assays

The Perfect Partner for Promega Assays

Preloaded Promega protocols
or customize your own

Cell Signaling & Metabolism Assays:
Including:
- ADP-Glo™
- Kinase-Glo®
- P450-Glo™
- cAMP-Glo™

Cell Health Assays:
Including:
- CellTiter-Glo®
- CellTox™ Green
- Caspase-Glo®
- BacTiter-Glo®

Luciferase Reporter Assays: Including:
- Nano-Glo®
- ONE-Glo™
- Dual-Glo® & DLR
- Bright-Glo™

BRET and FRET Assays: Including:
- NanoBRET™
- Renilla/YFP
- Commercial and Homebrew assays

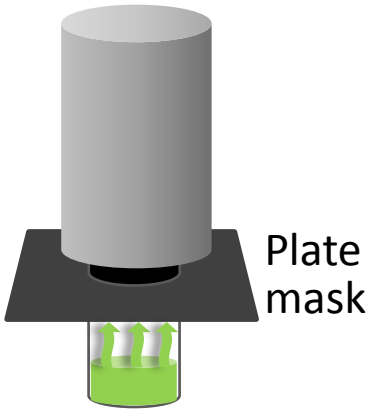
…plus many, many more

DLReady

NanoLuc® Luciferase Ready

Built-In Detectors for each Module
-behaves like three separate instruments

Head-on PMT for photon counting

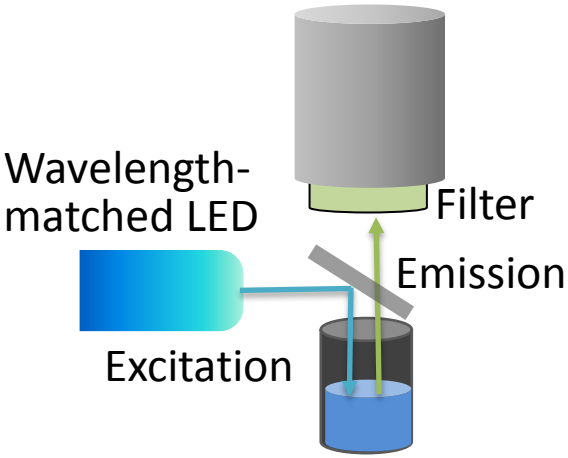


Luminometer

Plate mask switching for 96/384-well measurements

- $3x10^{-21}$ moles Luciferase sensitivity
- 9 logs dynamic range

PiN-Photodiode

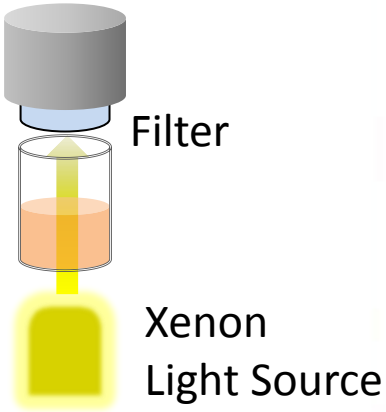


Fluorometer

Filter slides for automatic filter switching

- 2fmol/200ul fluorescein sensitivity
- 6 logs dynamic range

PMT



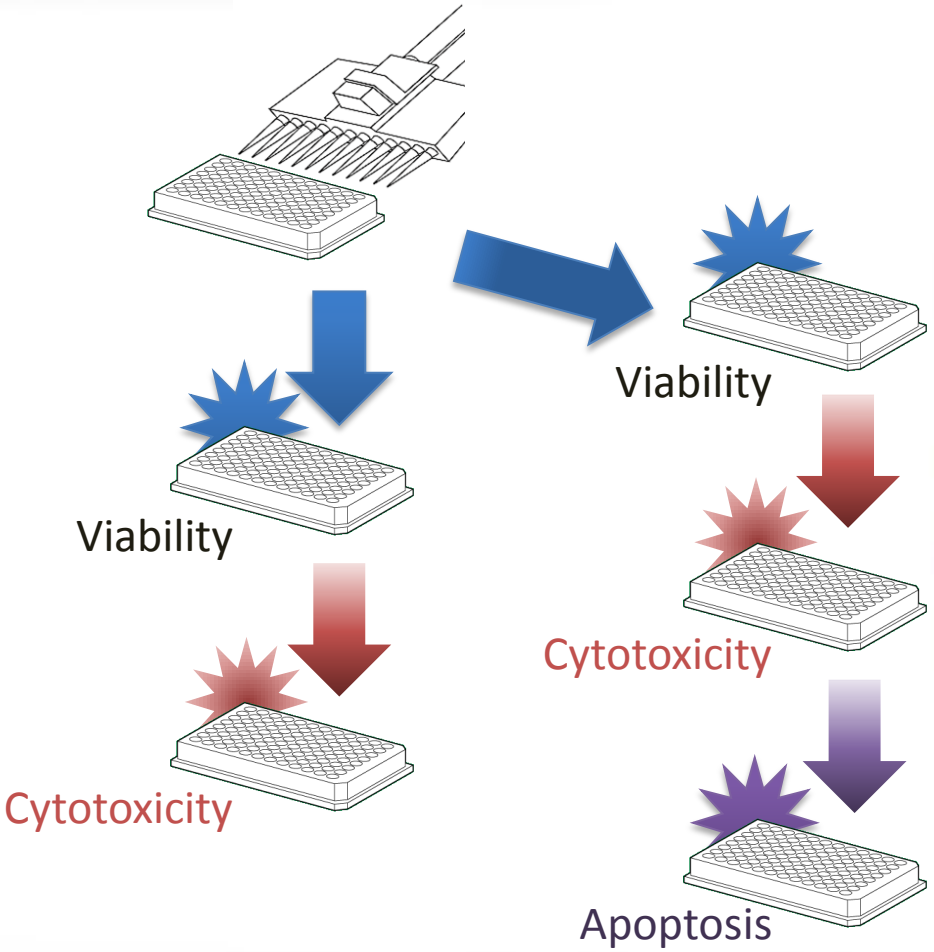
Photometer

Filter Wheel with UV-Vis Abs filters

- 0.01 OD sensitivity
- 0-4 OD range

Multiplex Assays for a More Complete Picture

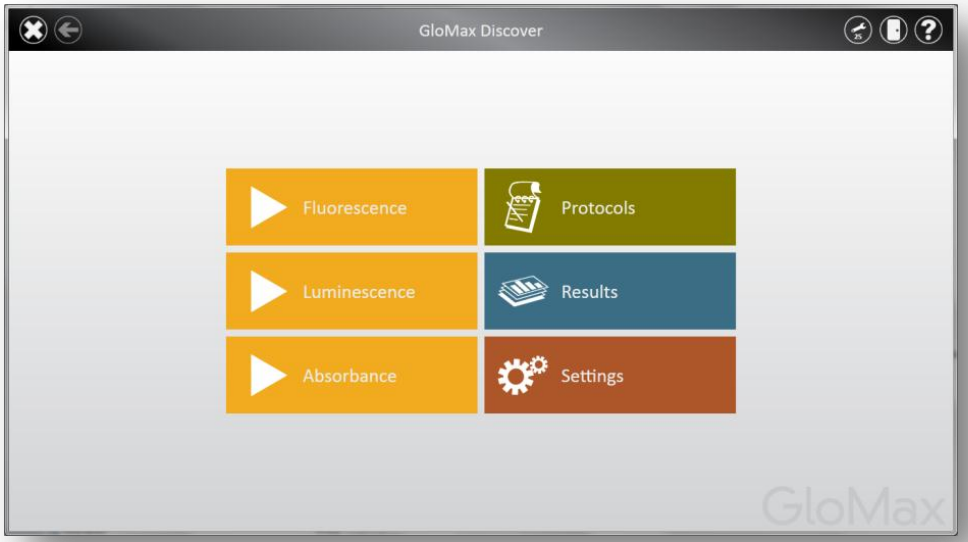
- Automatic filter slides enable multiple reads without user intervention
- Intuitive software provides flexible and easy protocol design
- Easily perform BRET and FRET studies
- Customize filters for your needs



Intuitive Software Makes it Easy

Simple GUI Interface

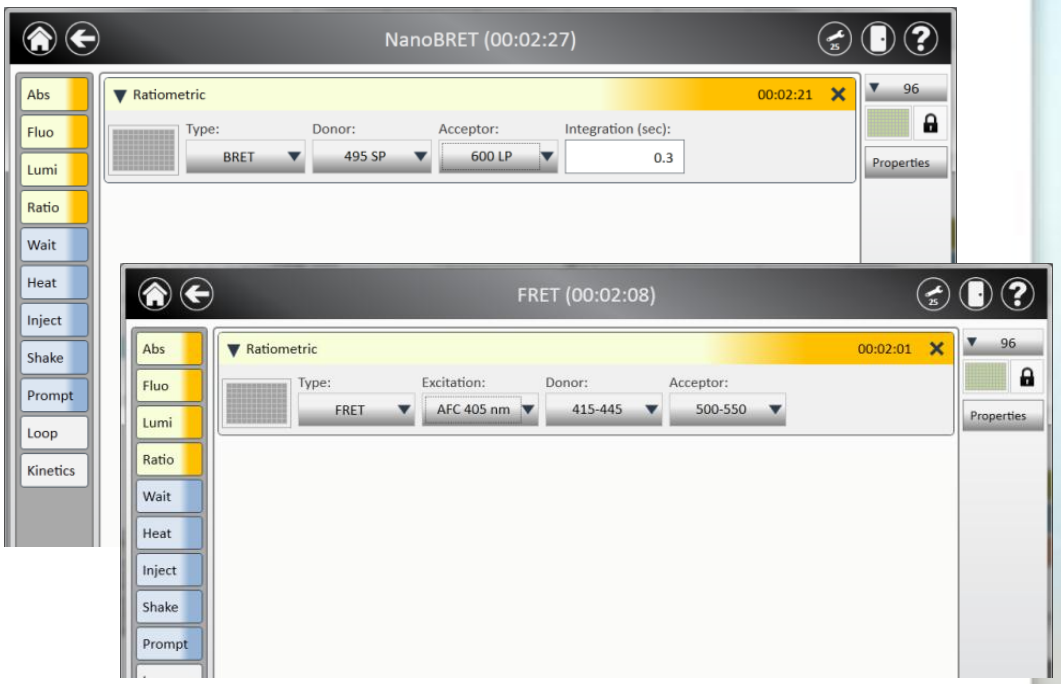
- Quick Start reads
- Select / Create Protocols
- View / Export Results
- User Settings



Intuitive Software Makes it Easy

Flexible protocol-builder

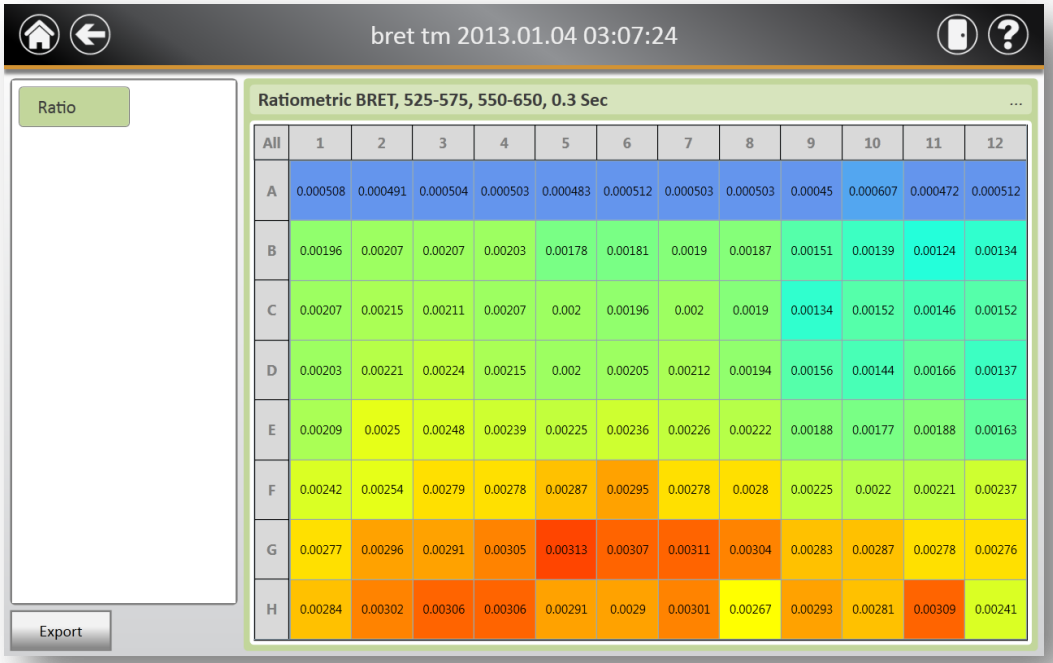
- User customizable
- State-of-the-art touchscreen response
- Drag and drop navigation



Intuitive Software Makes it Easy

Data Portability

- Network-ready
- Heat-map display
- Multi-touch pinch/zoom gestures
- Export to USB, microSD, network, LIMS, Cloud



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A	0.000508	0.000491	0.000504	0.000503	0.000483	0.000512	0.000503	0.000503	0.00045	0.000607	0.000472	0.000512
B	0.00196	0.00207	0.00207	0.00203	0.00178	0.00181	0.0019	0.00187	0.00151	0.00139	0.00124	0.00134
C	0.00207	0.00215	0.00211	0.00207	0.002	0.00196	0.002	0.0019	0.00134	0.00152	0.00146	0.00152
D	0.00203	0.00221	0.00224	0.00215	0.002	0.00205	0.00212	0.00194	0.00156	0.00144	0.00166	0.00137
E	0.00209	0.0025	0.00248	0.00239	0.00225	0.00236	0.00226	0.00222	0.00188	0.00177	0.00188	0.00163
F	0.00242	0.00254	0.00279	0.00278	0.00287	0.00295	0.00278	0.0028	0.00225	0.0022	0.00221	0.00237
G	0.00277	0.00296	0.00291	0.00305	0.00313	0.00307	0.00311	0.00304	0.00283	0.00287	0.00278	0.00276
H	0.00284	0.00302	0.00306	0.00306	0.00291	0.0029	0.00301	0.00267	0.00293	0.00281	0.00309	0.00241

An Extensive List of Applications on Promega.com

Application Notes: GloMax® Discover System

GloMax® Discover System

Bioassays

Measuring the ADCC Reporter Bioassay Complete Kit (WIL2-S) Signal on the GloMax® Discover System

Cell Health and Metabolism

Measuring the Output of the CytoTox-Fluor™ Cytotoxicity Assay on the GloMax® Discover System

Measuring the ONE-Glo + Tox Luciferase Reporter and Cell Viability Assay on the GloMax® Discover System

Measuring Cell Viability Using the CellTiter-Glo® Cell Viability Assay and GloMax® Discover System

Measuring P450-Glo™ Assays on the GloMax® Discover System

Measuring Bacterial Cell Viability Using the BacTiter-Glo™ Assay and GloMax® Discover System

Measuring Fluorescence Using the Apo-ONE® Homogeneous Caspase-3/7 Assay with the GloMax® Discover System

Measuring Fluorescence Using the CellTiter-Blue® Cell Viability Assay with the GloMax® Discover System

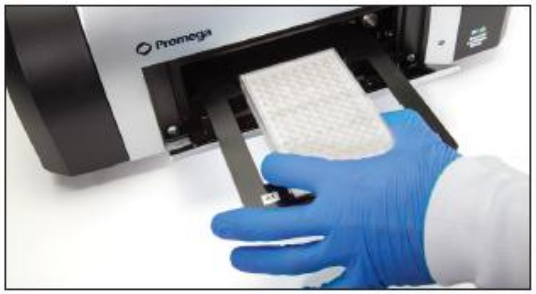
Measuring Fluorescence Using the ApoTox-Glo™

www.promega.com/discover

Using GloMax® Discover is Simple

1) Open door

2) Add plate (note: A1)



3) Select protocol

4) Select wells

5) Start read

6) Export results

What's Inside

To access the interior of the instrument:

1) Hold the door open

2) Use the Phillips screwdriver to unscrew the 2 screws (one at the left side, and one at the right side).

   Note: Only a ¼ turn is necessary

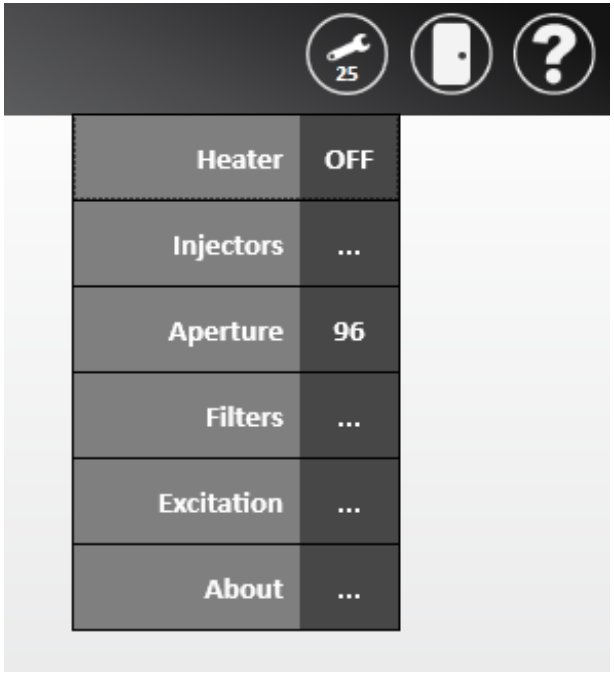
3) Pull the front access panel off

What's Inside

To change excitation filters, emission filters, or apertures:

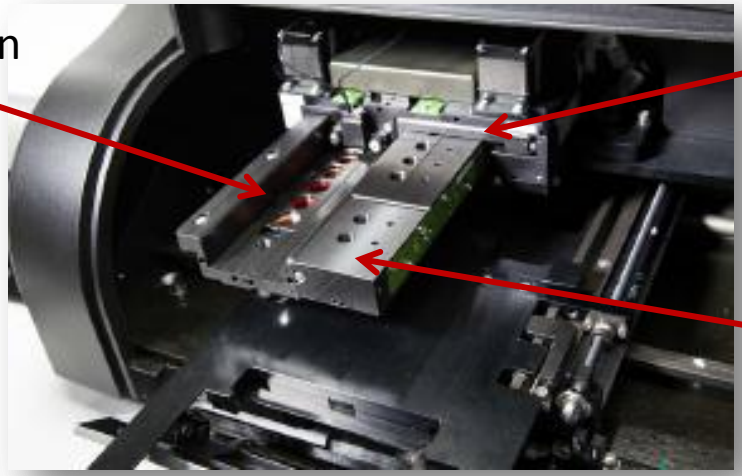
Touch the Tools icon at the top of the window and then the desired function

Follow the on-screen instructions



What's Inside

Luminescence Emission filters



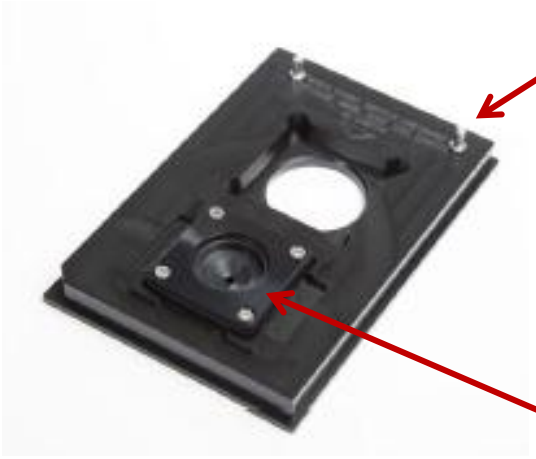
Fluorescence Emission filters

Fluorescence Excitation modules

What's Inside

Semi-automated method for changing the internal aperture.

1. Follow the on-screen guide to change the aperture
2. Make sure to load the Aperture Plate correctly



Fluorescence Filters

5 Standard Excitation filters and 2 custom positions.

Empty Emission filter paddles for 6 customized positions are available as accessory

	Excitation Peak Wave length	Emission wave length	Assays
UV	365	415-445	Hoechst dye, 4-MU
Blue	475	500-550	EGFP, or hMGFP, DNA, RNA or protein quantitation dyes, QuantiFluor™ , Fluorescein, Rhodamine-110
Green	525	580-640	Rhodamine, Cy®3, resorufin
Red	605	660-720	Cy®5, RNA quantitation dyes
AFC	405	495-505	Aminofluorocoumarin

Absorbance Filters

9 UV-Vis Absorbance filters are provided with the instrument

The wavelength just needs to be close, not exact. A 450nm filter is actually 445-455nm

i.e. Pierce 660 Reagent calls for a 660nm filter, but 600nm works great, that's 30nm off-peak

Filters (10nm bandpass)	Assays
230nm	Contaminants (Guanidine, Phenol, carbohydrates)
260nm	Nucleic Acid Quantitation
280nm	Protein Quantitation (Nucleic Acid Purity)
320nm	Background subtraction for Nucleic Acid/Protein Quantitation
405nm	Colorimetric Promega Assays (CaspACE)
450nm	ELISA Assays
490nm	Colorimetric Promega Assays (CellTiter 96/Aqueous/Aqueous ONE / CellTiter Blue)
560nm	BCA Protein Assays
600nm	Bradford Protein Assays Coomassie® Blue Protein Assays

Luminescence Filters

Luminescence Filters (standard)

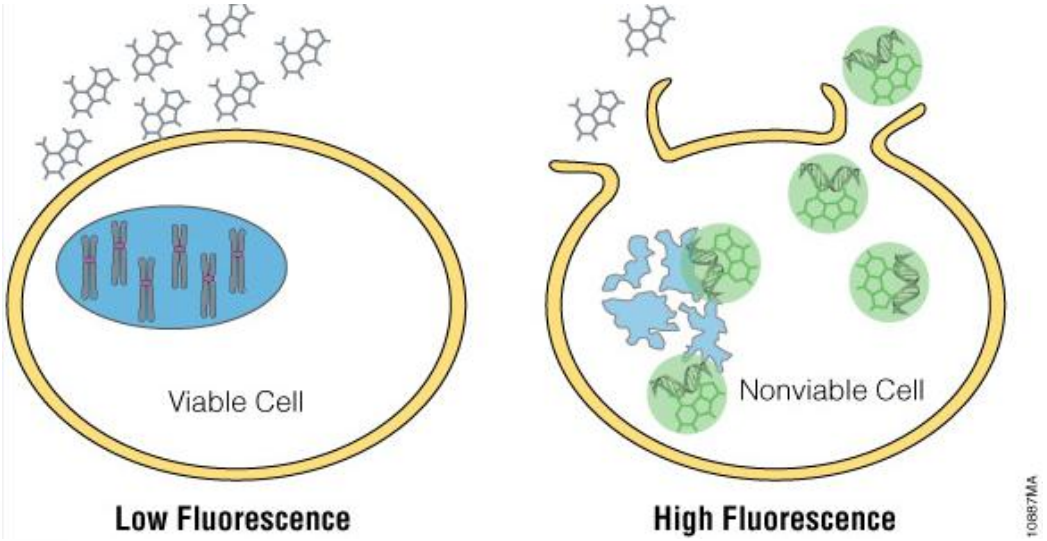
Position	Filter wavelength	Assay
5	Empty (user configurable)	
4	530nm LP	Click Beetle Luciferase; BRET1
3	540nm SP	Firefly Luciferase; ChromaGlo
2	600nm LP	HaloTag
1	495nm SP	NanoLuc (NanoBRET), Renilla Luciferase

- All of the filters needed for NanoBRET™ are included
- No additional custom filters are needed for NanoBRET™

Assay performance

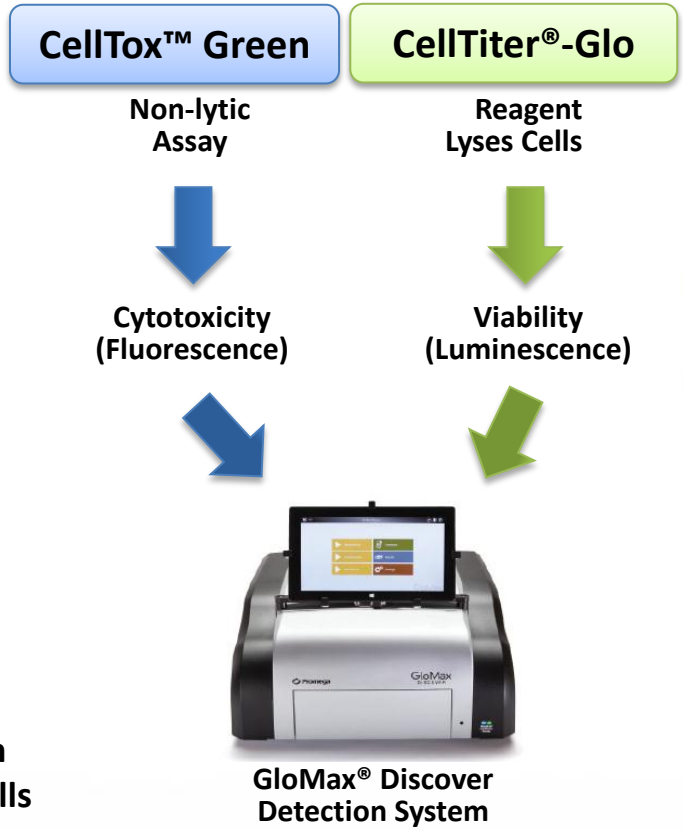
CellTox™ Green for Real-Time Cytotoxicity

- Measures changes in membrane integrity as a result of cell death
- Dye preferentially stains dead cell DNA
- Seamless multiplex with viability assay to provide mechanistic information related to cytotoxicity



Excluded dye yields no increase in fluorescence with viable cells

Non-excluded dye yields increase in Fluorescence with compromised cells



CellTox™ Green for Real-Time Cytotoxicity

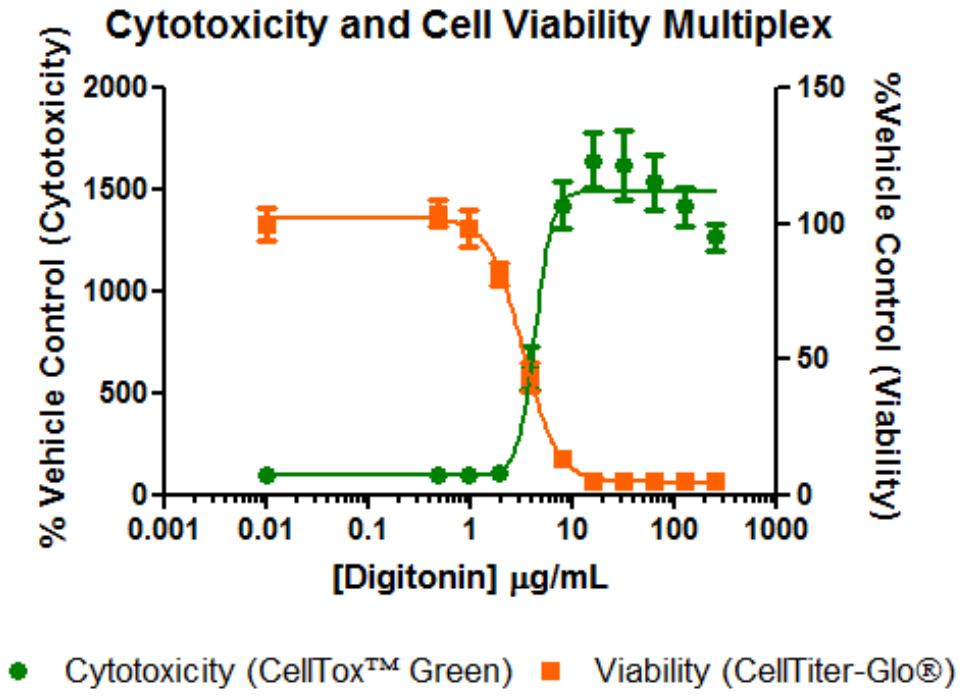
Expected Results:

- A dose-dependent effect on K562 cell viability. As digitonin concentration increase, so does cytotoxicity

- Luminescent ATP detection decreases due to decreased cell viability

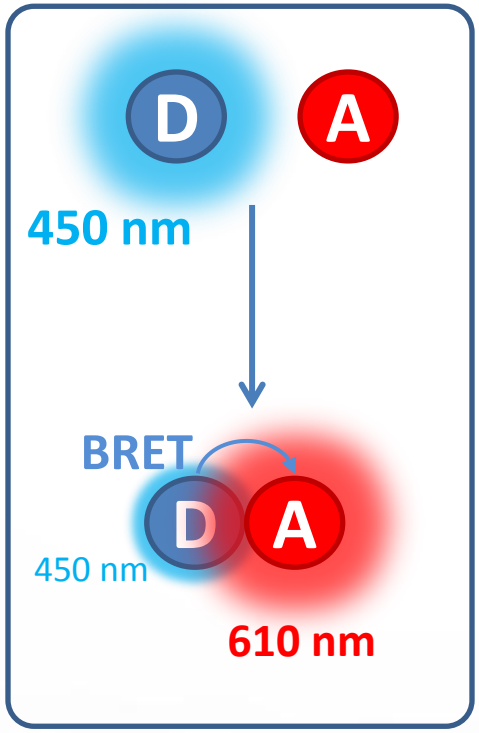
- GloMax® Discover provides easy setup to multiplex Promega assays

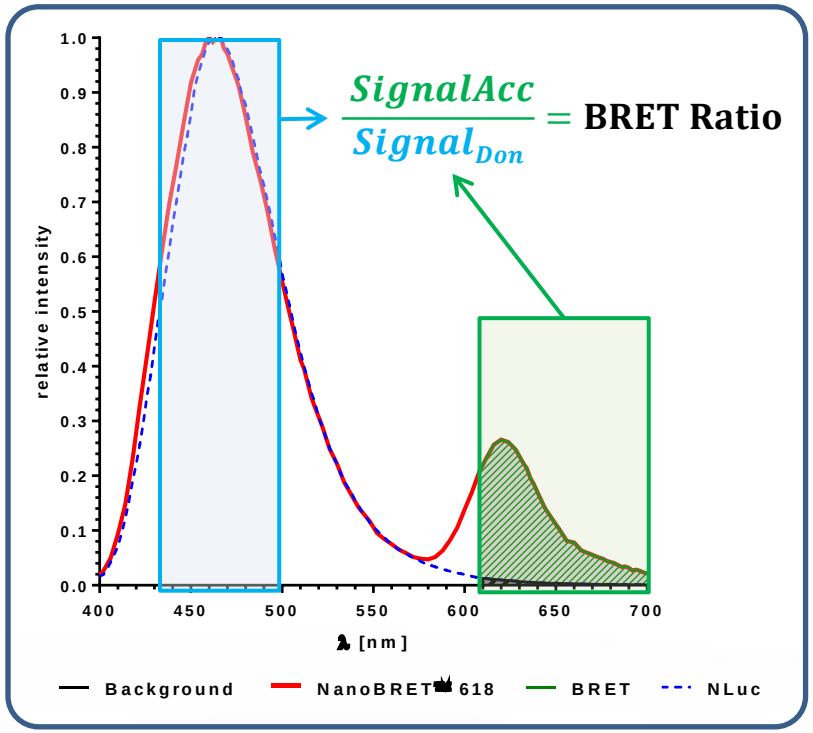
- GloMax® Discover results provide expected biology



NanoBRET™ Technology for Protein Interactions

- BRET provides real time measurement *in living cells*
- Superior luminescent Donor signal from NanoLuc™
- Flexible choice of Donor/Acceptor Separation
- Low Donor/Acceptor ratios provides best dynamic range





NanoBRET™

Express Donor and Acceptor protein fusions

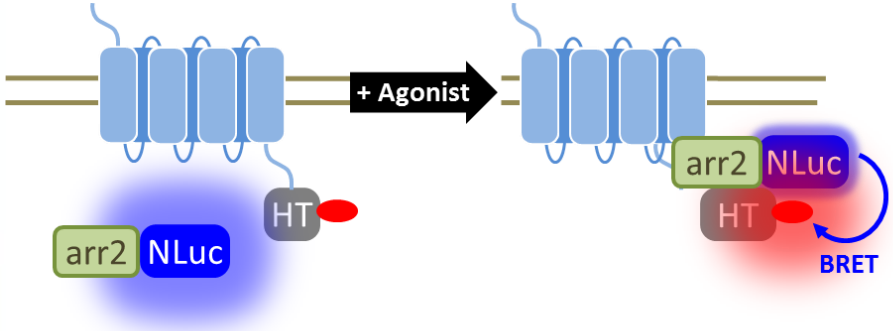
Label Cells with HaloTag (Acceptor fusion)

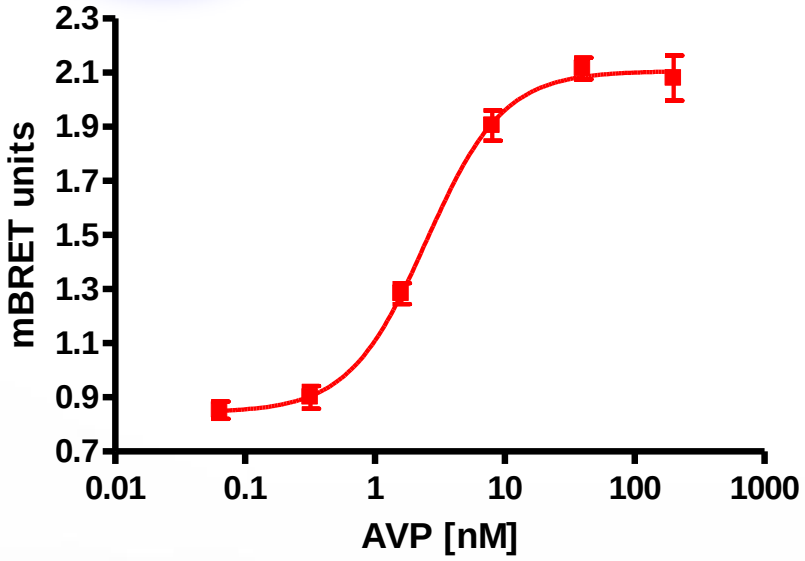
Induce interaction

GloMax® Discover Detection System

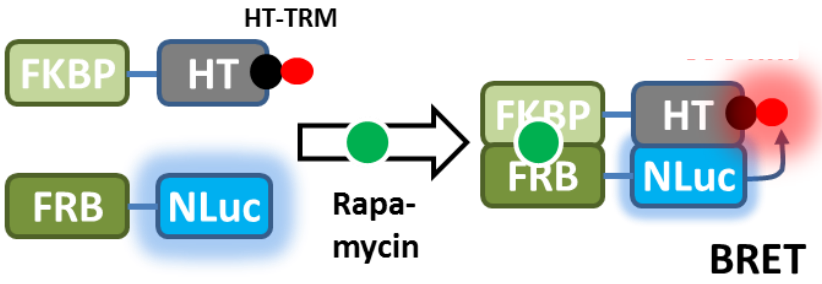
NanoBRET™ Application Examples

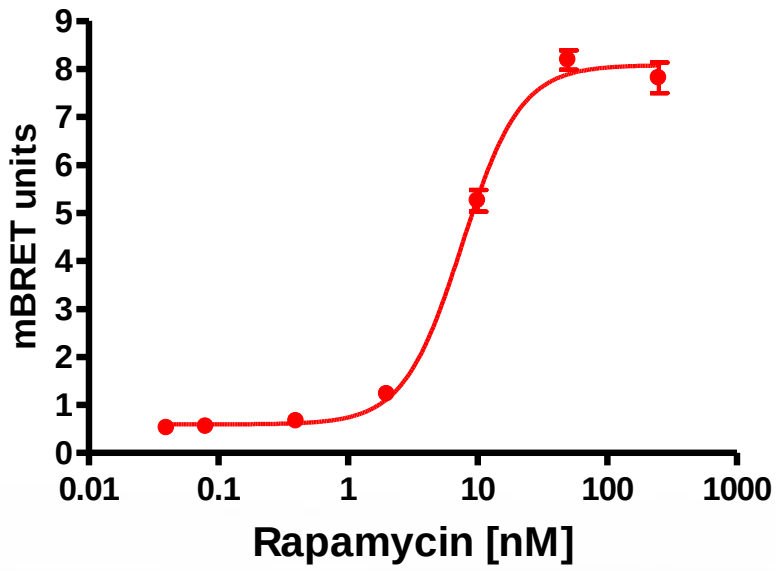
AVPR2 / β-arr2 Model





FKBP/Frb/Rapamycin Model

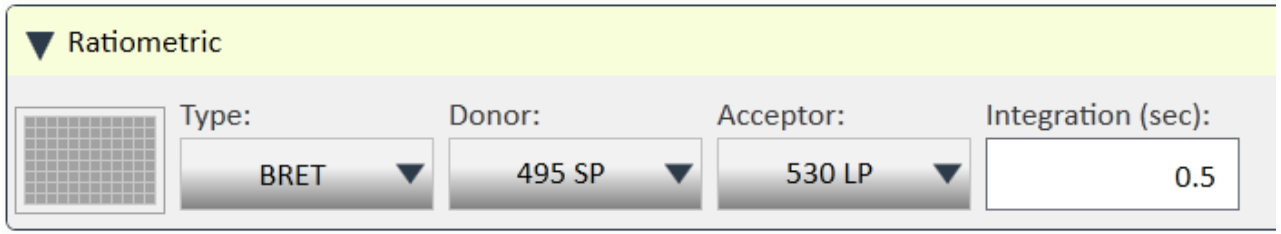




Other BRET and Filtered Luminescence Assays

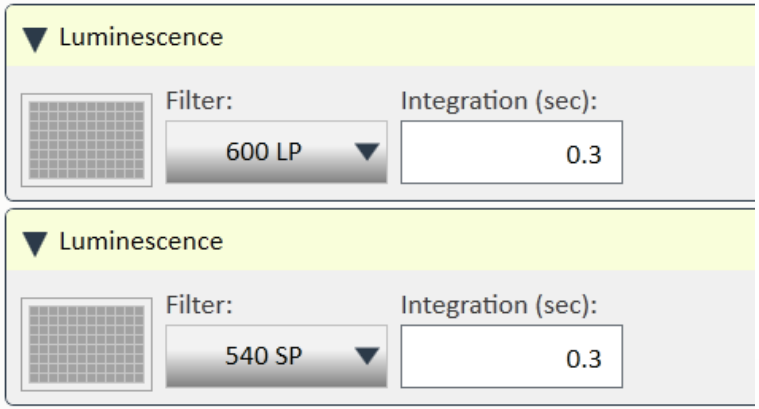
Renilla / YFP

Donor Filter	Acceptor Filter
495nm SP	530nm LP



Chroma-Glo

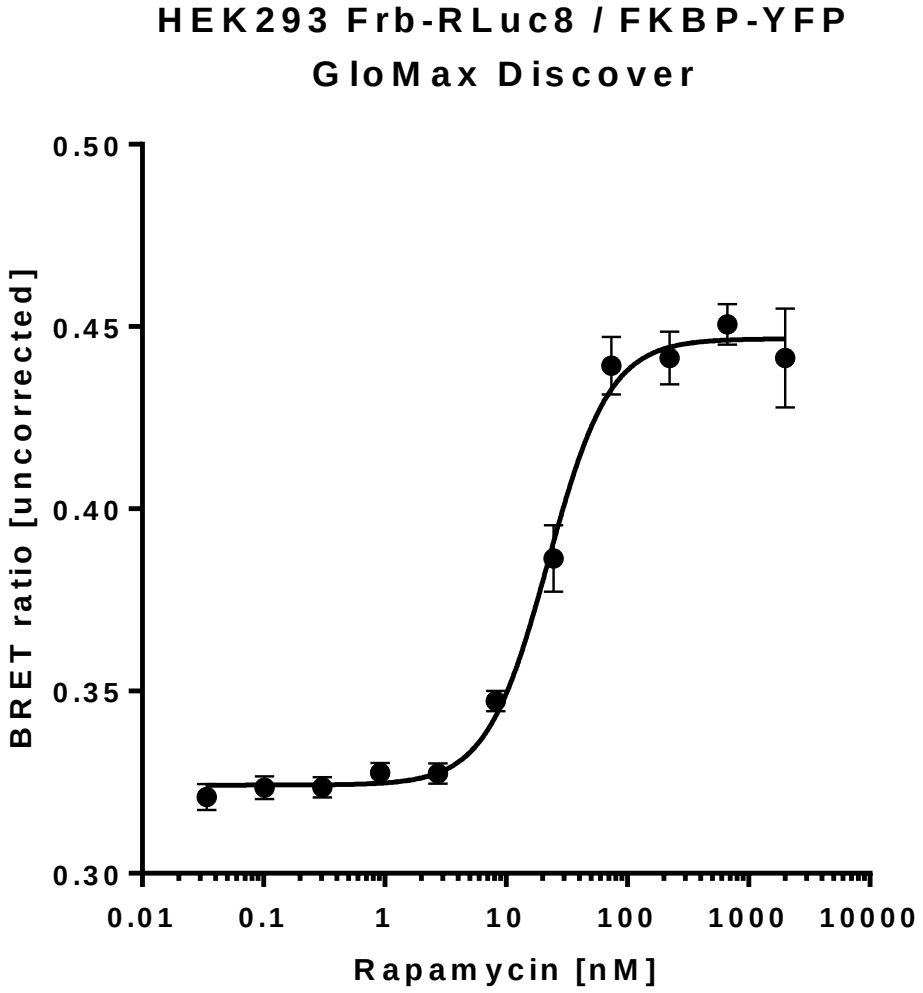
1st reading	2nd reading
600nm LP	540nm SP



BRET1: Frb-RLuc8 / FKBP-YFP

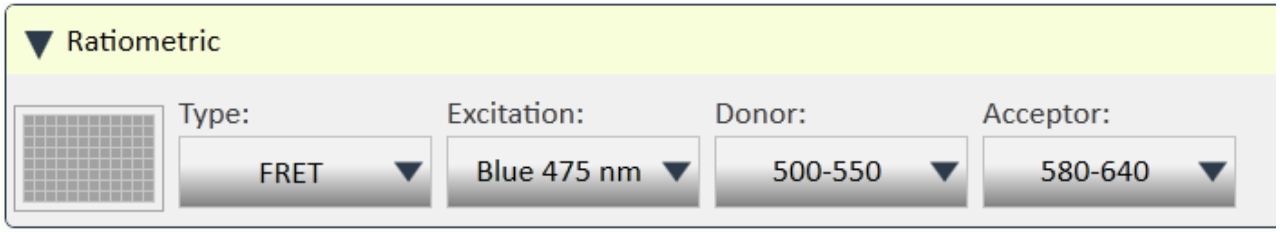
Experiment

- HEK293 cells reverse transfected with expression constructs for Frb-RLuc8 and FKBP-YFP (ratio 1:4) using Fugene HD
- Cells were plated in white 96-well plates in DMEM.
- After 24 h medium was replaced with Optimem.
- Cells were treated with a serial dilution of Rapamycin for 15 minutes at 37C before Coelenterazine h was added to a final concentration of 20 uM.
- Plate was read in GloMax Discover using the following settings
- Donor filter 495 shortpass filter
- Acceptor filter 530 nm longpass filter
- 0.5 seconds integration time

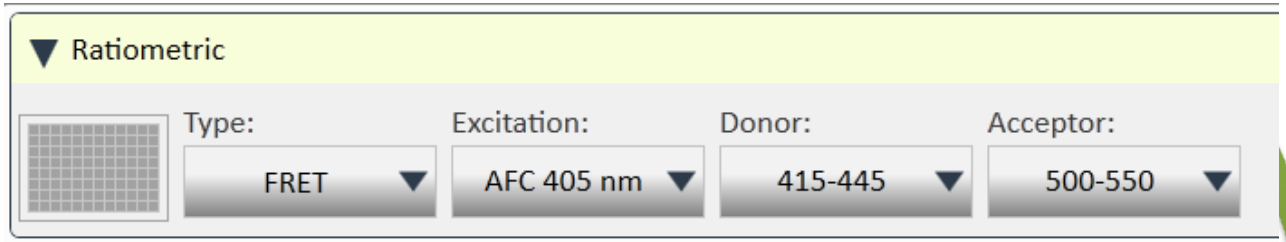


FRET Assays

GFP / RFP FRET
Fluorescein / Rhodamine FRET



Coumerin / Fluorescein FRET



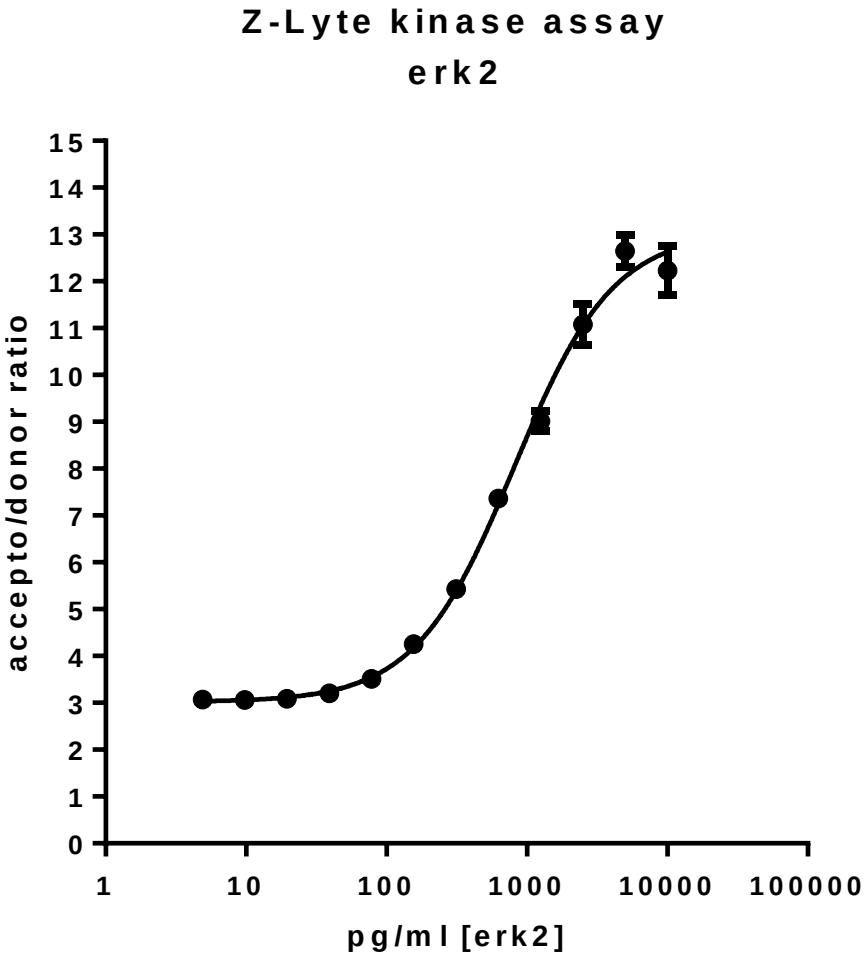
Primary Excitation	Primary Emission	Secondary Excitation	Secondary Emission	Application
475nm	509nm	563nm	582nm	GFP / RFP
447nm	520nm	550nm	573nm	Fluorescein / Rhodamine
409nm	447nm	447nm	520nm	Coumerin / Fluorescein; Life Technology Z-Lyte Assay

FRET: Z-Lyte kinase assay for erk2 activity

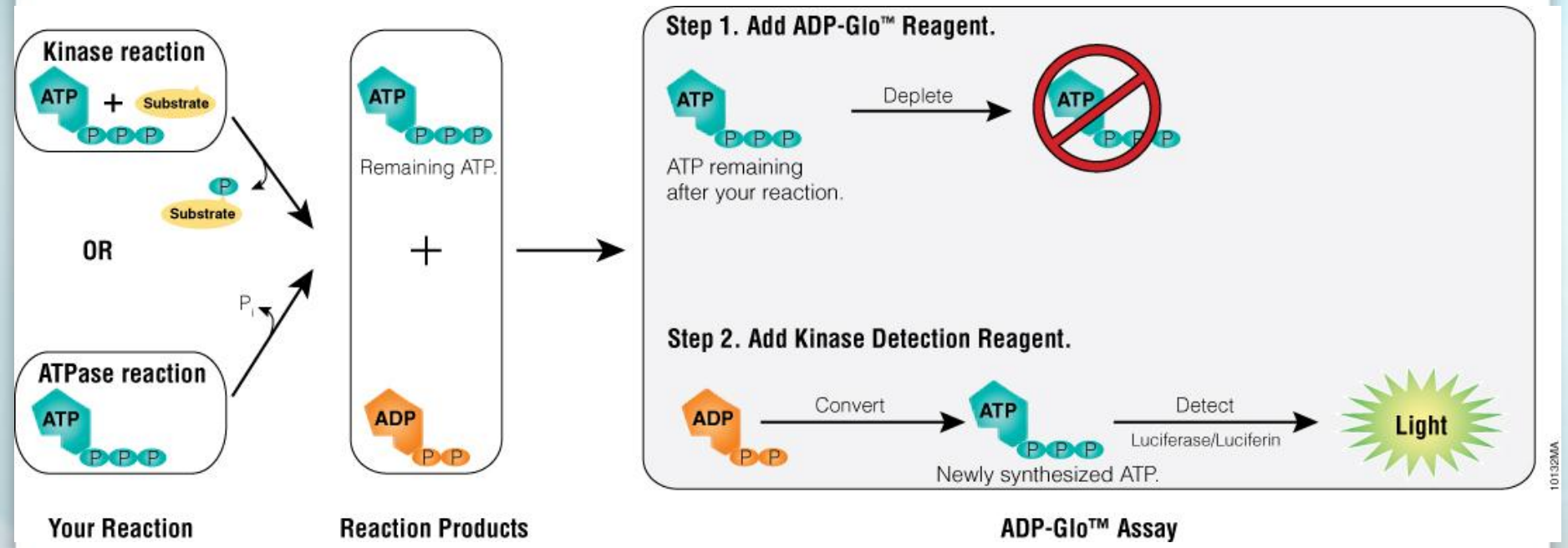
Experiment

- Erk2 kinase activity was determined using the Z-Lyte Kinase assay (Life Technologies) according to manufacturers instructions
- ATP = 0.5 mM
- Activity measurement was taken in a GloMax Discover using the following filter settings

Excitation:	**AFC 405 nm**
Donor:	**415-445**
Acceptor:	**500-550**



ADP-Glo™ Assay and Kinase Profiling Systems



- Measures kinase activity in any purified kinase enzyme
- Direct correlation of kinase activity to luminescence output
- Sensitive and suitable for studying kinases in physiological conditions

ADP-Glo™ Assay and Kinase Profiling Systems

Kinase profiling System – ST-5 Kit components

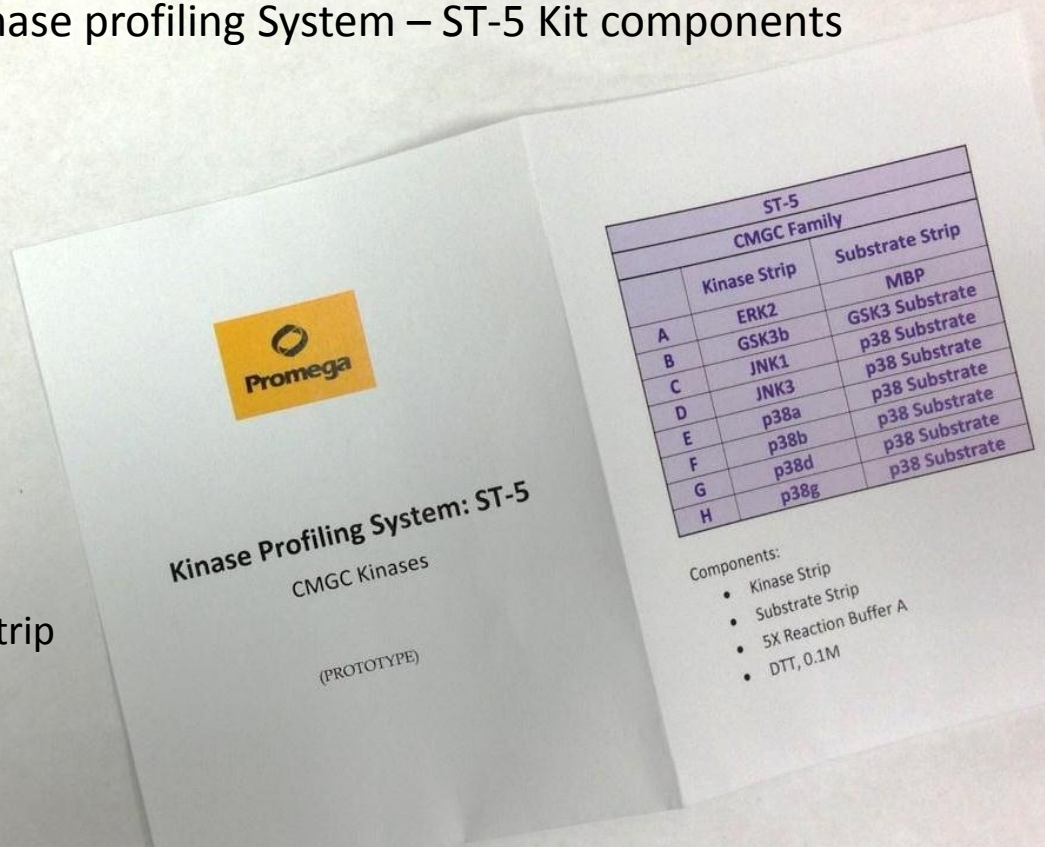
2 strips

2.5µL Kinase Strip, 50X
(some strips may contain 5µl)

2 strips

37.5µL Substrate/Co-Factor Strip

1.5mL 5X Reaction Buffer A
25µL DTT, 0.1M



Kinase Selectivity Profiling

384-well plate

5µl kinase reaction

+

5µl ADP-Glo™ Reagent

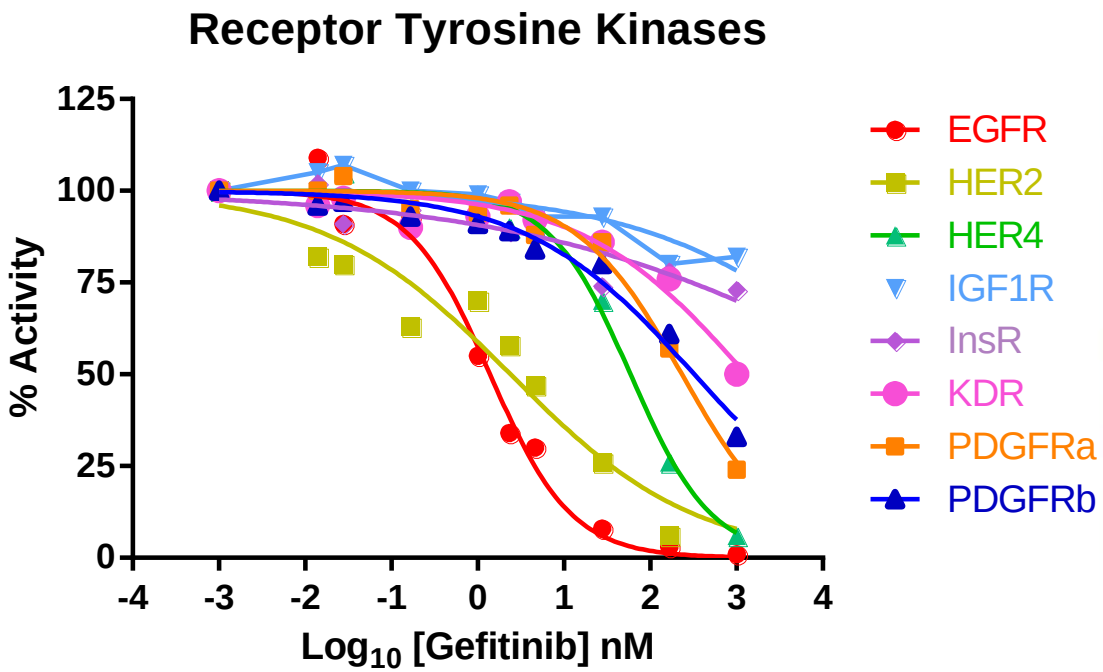
40 min. Incubation

+

10µl Kinase Detection Reagent

30-60 min. Incubation

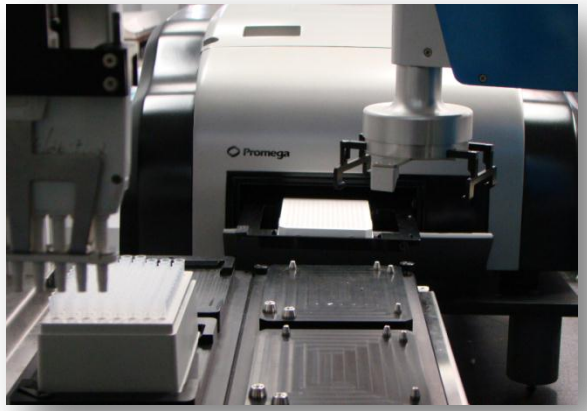
Record Luminescence



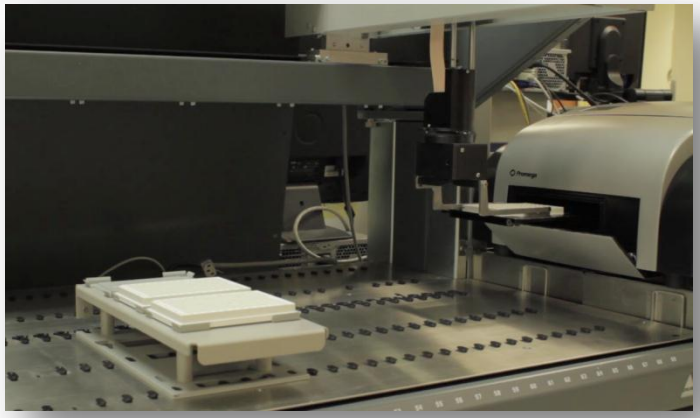
Automation Integration

Demonstrated Integration with:

- **Tecan** Freedom EVO® liquid handler
- **Hudson Robotics** Solo™ liquid handler and PlateCraneEX™ robotic arm
- Additional platforms planned



3rd party software control of Discover



Data CSV format for **LIMS data** integration

Integrator's Kit (PDF command-line instructions)

GloMax® Discover is Designed to be SiLA Compliant

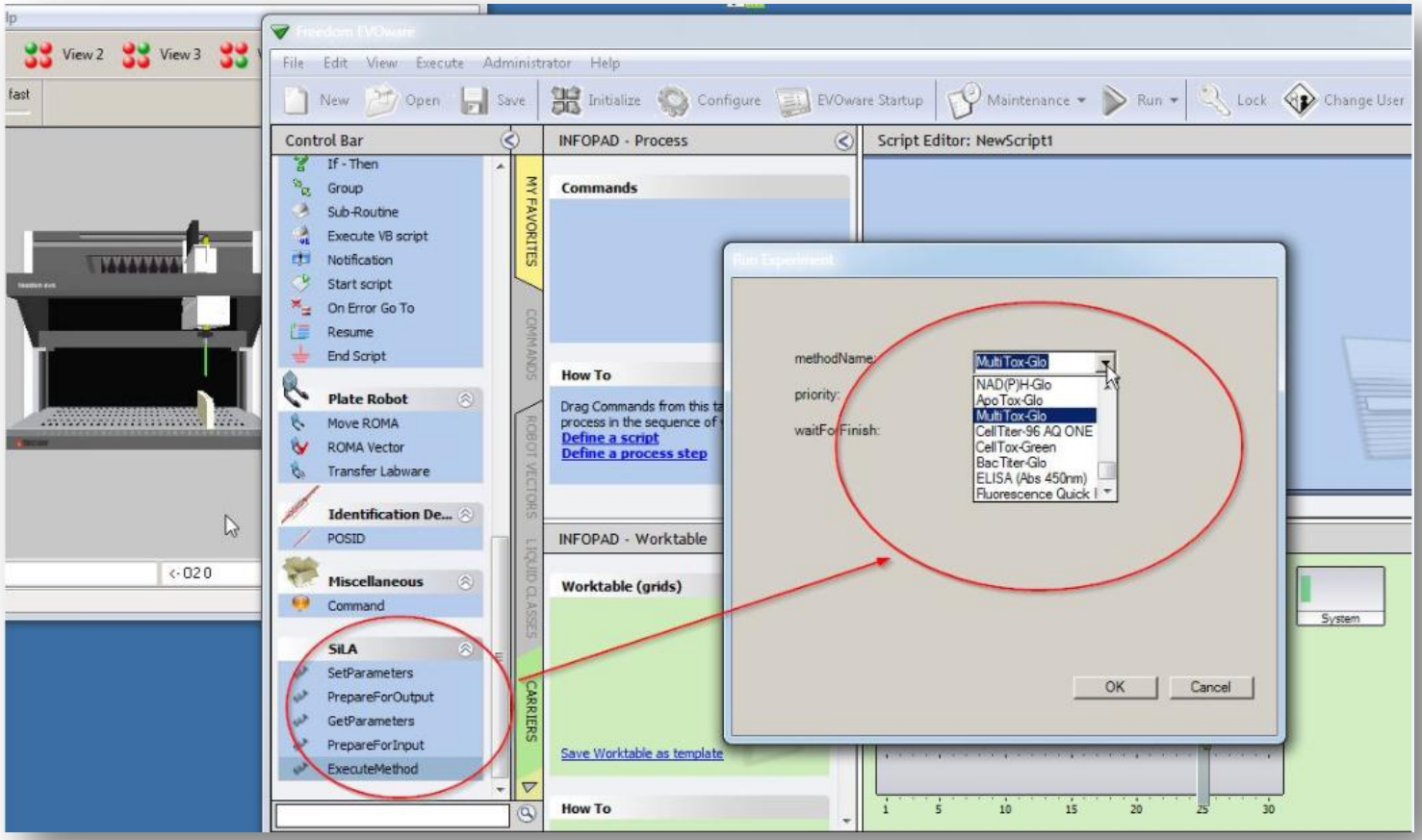
What is SiLA? (Standards in Laboratory Automation)

- A new industry standard to standardize
  - Device Control and Interfaces
  - Data Capture, Labware, etc.
- Avoids the need for custom software drivers when integrating devices

SiLA Rapid Integration®

GloMax® Discover SiLA Driver Available in 2014

Demonstrated with Tecan EVOWare® 2.6



Promega's SiLA Driver will be available in 2014

GloMax® Discover

Integrated	Seamless workflow with Promega Cell and Reporter assays.
Performance	Broad dynamic range, high sensitivity, and low well-to-well cross-talk for more usable data from your experiment.
Easy-to-use	Simple Tablet PC touchscreen navigation with full PC capabilities and a state-of-the-art Graphical User Interface makes the workflow simple, smooth, yet flexibly. Auto-gain adjustment so end-users don't have to worry about it.
Connected to your Workflow	Stand-alone instrument or integrate with automation. Export data to your laboratory network, LIMS, or Cloud. 21CFR Part 11 electronic signature compliant as standard.

Additional information

Request a free demo at
www.promega.com/discover