
Promega
Live-Cell Kinetic Assays
for Time-Course Analysis
Cell Viability | Apoptosis | Cytotoxicity
Extracellular ATP | Reporter Gene Detection

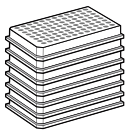
# Transform Your Time-Course Into a One-Plate Experiment

## Real-Time Assay

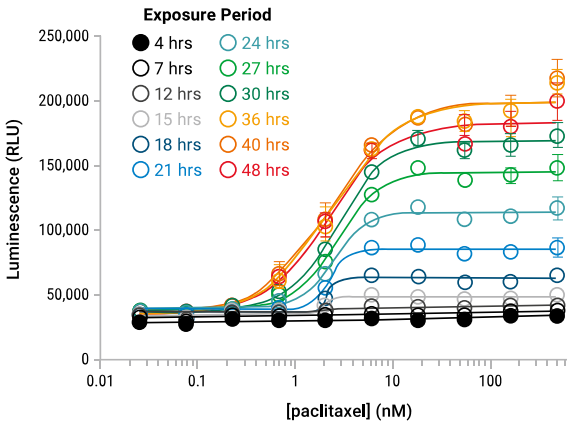
1 Plate

vs.

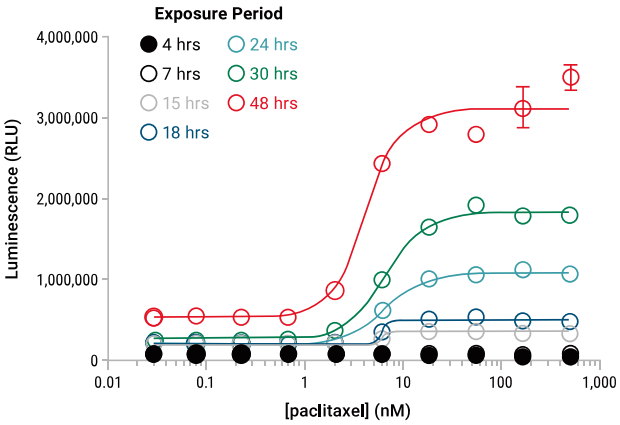
## Endpoint Assay



7 Plates




Multiple data points. **One assay plate.**




Multiple data points. **Multiple assay plates.**

**Left panel: Real-time assay.** Data for each dose was obtained at multiple time points using a single assay plate with one **RealTime-Glo™ Annexin V Apoptosis Assay** Reagent addition.

**Right panel: Endpoint assay**. Seven separate plates were used to collect parallel data using an endpoint assay.

# Why Use Live-Cell Kinetic Assays?

Live-cell kinetic assays allow the same sample well to be repeatedly measured over multiple time points. **This saves you time and effort, enabling you to collect more informative data in real-time.**

## Live-Cell Kinetic Assay Benefits

### *No more missing time points:*

Most endpoint assays lyse the cells to collect data only from a single time point. Live-cell kinetic assays are non-lytic and non-toxic. The cells remain viable, which allows you to detect kinetic changes over a longer time.

### *Save time, cells, and reagents:*

Perform an extended time study with a single plate, instead of the need to have separate plates for each time point. A single-plate assay uses fewer cells and plates, also less culture media and other cell culture tools than multiple plates.

### *More data from one well with less variability:*

Live-cell kinetic assays are non-lytic. This allows additional downstream applications, including multiplexing with other assays to gain more data from a single assay plate. The use of multiple assays from the same cells reduces assay variability in comparison to working with cells in multiple replicate plates.

## Live-Cell Kinetic Assay Workflow

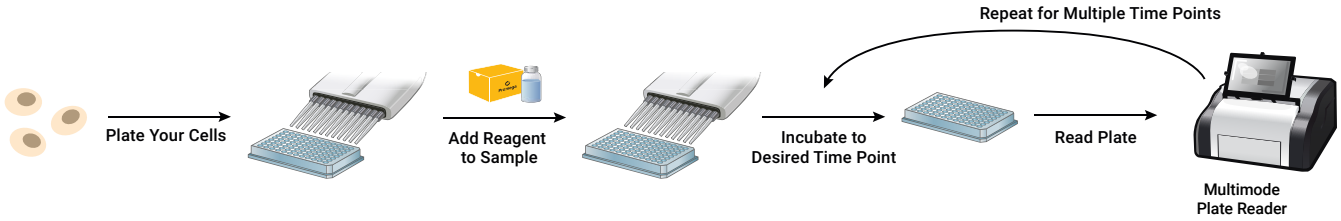



Plate your cells and add the reagent directly to the cells only once. The assay can be performed without further washing or processing steps. Read the plate at each time point, as many times as you need!

# Monitoring Cell Health in Real-Time

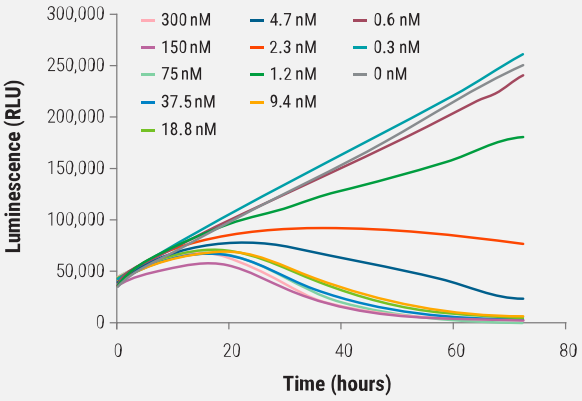
## Live-Cell Kinetic Assays for Cell Viability, Apoptosis & Necrosis, Cytotoxicity, and Immunogenic Cell Death Measurement

### Assay Features

- Continuous monitoring of the cell state
- Non-lytic, plate-based, and scalable
- Compatible with 3D culture models
- Multiplexing compatible

### RealTime-Glo™ MT Cell Viability Assay

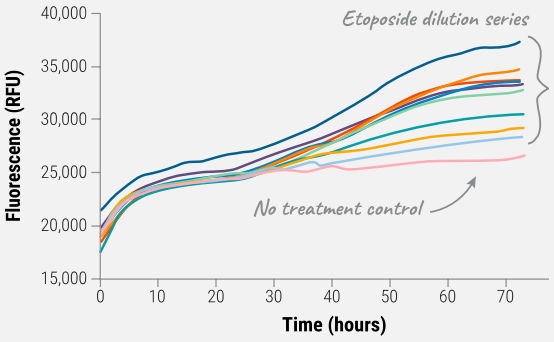
The RealTime-Glo™ MT Cell Viability Assay is a homogeneous, bioluminescent method to determine in real-time the number of viable cells in culture by measuring the reducing potential of cells. The reagent is stable for up to 72 hours. No cell washing, removal of medium, or further reagent addition is required. The assay can be multiplexed with other assays, e.g., the CellTox™ Green Cytotoxicity Assay, to collect more informative data from the same well.




A549 cells (500 cells/well) were plated in medium containing the RealTime-Glo™ Reagent in 384-well plates. Cells were treated with bortezomib at the indicated concentrations and monitored every hour for 72 hours.

### CellTox™ Green Cytotoxicity Assay

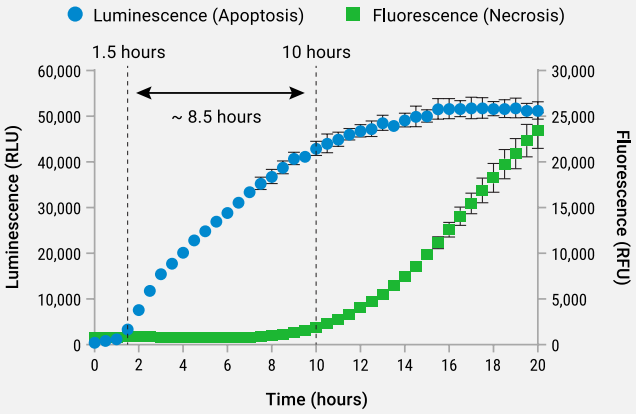
The CellTox™ Green Cytotoxicity Assay measures changes in membrane integrity that occur as a result of cell death. The assay system uses a proprietary asymmetric cyanine dye that is excluded from viable cells but preferentially stains the DNA from dead cells. The fluorescent signal remains constant after exposure of 72 hours. The assay can be multiplexed with other spectrally distinct cell health assays.




MCF7 cells (500 cells/well) plated in medium containing the CellTox™ Green Reagent were treated with a dilution series of etoposide (0—250 µg). Cytotoxicity was measured from the same sample well every hour for 72 h.

## RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay

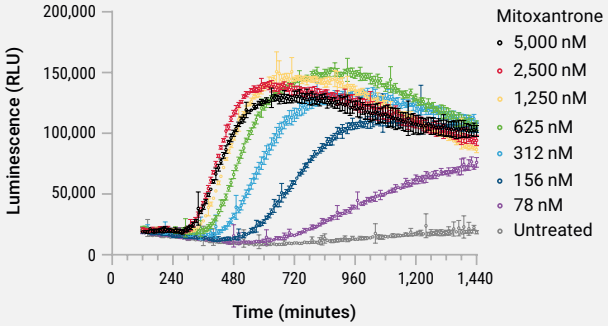
The RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay measures the real-time exposure of phosphatidylserine (PS) on the outer leaflet of cell membranes during the progression of apoptosis. The assay also includes a cell-impermeant, profluorescent DNA dye, which detects necrosis. This simple "add-read-assay" allows to perform real-time kinetics of up to 48 hours.




DLD-1 cells treated with 400 ng/ml rhTRAIL as extrinsic inducer of apoptosis. A time delay between the emergence of PS:Annexin V binding (luminescence) and the loss of membrane integrity (fluorescence) of 8.5 hrs indicate an apoptotic phenotype followed by secondary necrosis.

## RealTime-Glo™ Extracellular ATP Assay

The RealTime-Glo™ Extracellular ATP Assay can be used for kinetic monitoring of ATP release from dying, stressed, or activated cells. Extracellular ATP (eATP) functions as a Damage Associated Molecular Pattern (DAMP) signal and is a key biomarker to determine whether a treatment induces Immunogenic Cell Death. The assay is based on an ATP-dependent luciferase reaction and allows real-time kinetics for up to 24 hours.




U937 cells were treated with a dilution of mitoxantrone, an anthracycline shown to induce Immunogenic Cell Death. RealTime-Glo™ Extracellular ATP Assay Reagent was added, and luminescence was collected every 10 minutes for 24 h.

## Cell Health Assays Summary

| Assay | Marker | Protocol Type | Plate Format | Instrument |
|---|---|---|---|---|
| RealTime-Glo™ MT Cell Viability Assay | Reducing potential of the cells | Endpoint or continuous readout up to 72 h | 96/384/1536 | Luminometer |
| CellTox™ Green Cytotoxicity Assay | DNA | Endpoint or continuous readout up to 72 h | 96/384/1536 | Fluorometer |
| RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay | PS, DNA | Continuous readout up to 48 h | 96/384/1536 | Luminometer (Apoptosis), Fluorometer (Necrosis) |
| RealTime-Glo™ Extracellular ATP Assay | eATP | Continuous readout up to 24 h | 96/384 | Luminometer |

*For more information download our brochure: www.promega.com/CellbasedAssays*

# Monitoring Bioluminescent Reporter Activity in Real-Time

## Live-Cell Detection of NanoLuc® Reporter Activity

Real-time monitoring of reporter activity provides an option to better understand the kinetics of a wide variety of cellular responses. The NanoLuc® luciferase provides a small, sensitive bioluminescent reporter that is compatible with real-time monitoring of gene expression events. Promega offers a collection of NanoLuc® reporter vectors into which you can clone your region of interest, and ready-to-use NanoLuc® reporter vectors for monitoring signaling pathways.

The Nano-Glo® Live-Cell Detection Reagents enable the kinetic measurement of NanoLuc® reporter activity. The reagents differ in their signal brightness and stability and are thus suitable for a variety of experimental goals:

### Nano-Glo® Live Cell Assay System

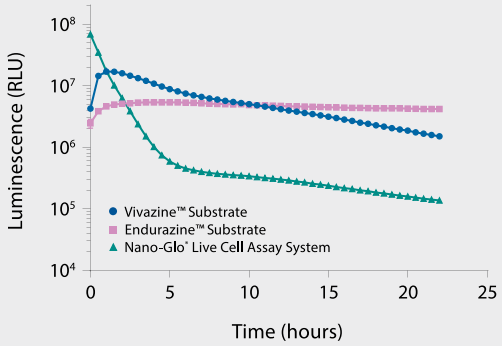
The Nano-Glo® Live Cell Assay System is a single-addition detection reagent, which offers the brightest signal and widest dynamic range for experiments less than 2 hours.

### Nano-Glo® Endurazine™ Live Cell Substrate

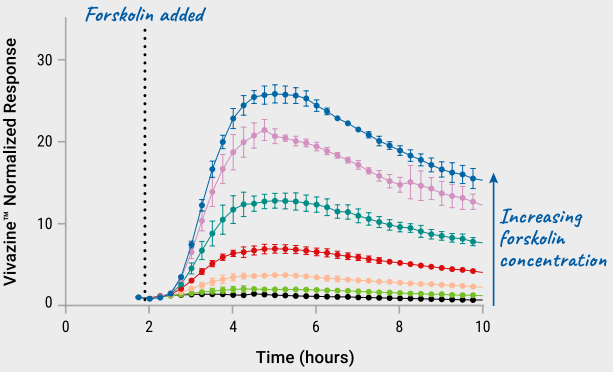
The Nano-Glo® Endurazine™ Live Cell Substrate provides the lowest initial signal intensity but has maximal stability for multi-day experiments.

### Nano-Glo® Vivazine™ Live Cell Substrate

The Nano-Glo® Vivazine™ Live Cell Substrate is an intermediate option with increased initial brightness but also increased rate of signal decay compared to the Endurazine™ Substrate.




Comparison of Endurazine™ and Vivazine™ Substrates with the Nano-Glo® Live Cell Assay System for signal intensity and stability. A plasmid encoding NanoLuc® luciferase expressed from the PGK promoter was transiently transfected into HEK293 cells and luminescence signal intensity was measured as indicated.




HEK293 cells were transiently transfected with a construct expressing NlucP from a promoter with cAMP response elements (CRE). Vivazine™ Substrate was added to cells at time zero, and luminescence was measured continuously. After 2 h, varying concentrations of forskolin were added to induce expression of NlucP by increasing the intracellular cAMP concentration. The graph shows a 10 h time-course with per-well normalization of the data.

*For more information about Nano-Glo® Live-Cell Reagents, visit: www.promega.com/LiveCellSubstrates*

# Ordering Information

| Live-Cell Cell Health Assays | Quantity | Cat. # |
|---|---|---|
| RealTime-Glo™ MT Cell Viability Assay | 100 assays | G9711 |
| CellTox™ Green Cytotoxicity Assay | 10 ml | G8741 |
| RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay | 100 assays | JA1011 |
| RealTime-Glo™ Extracellular ATP Assay | 200 assays | GA5010 |
| **Live-Cell Detection Reagents** | **Quantity** | **Cat. #** |
| Nano-Glo® Live Cell Assay System | 100 assays | N2011 |
| Nano-Glo® Endurazine™ Live Cell Substrate | 1 ml | N2571 |
| Nano-Glo® Vivazine™ Live Cell Substrate | 1 ml | N2581 |

*Number of assays refers to a 96-well plate format.*

*To learn more about Promega Live-Cell Kinetic Assays, visit: www.promega.com/LiveCellKineticAssays*

**Promega GmbH**
Gutenbergring 10
69190 Walldorf
www.promega.com

**Germany/Austria**
Phone +49 6227 6906-290
de_techserv@promega.com

**Poland**
Phone +48 22 531 0668
pl_techserv@promega.com

**Central/Eastern Europe**
Phone +49 6227 6906-0
de_ceesales@promega.com

REG4587