

Proteasome-Glo™ Cell-Based Assays For Chymotrypsin-, Trypsin- or Caspase-Like Activities

Benefits:

- **More Biologically Relevant Results:** Obtain proteasome activity data directly from a cellular environment.
- **Simplified Method:** The “Add-Mix-Read” protocol requires no lysate preparation, cell washing, media changes or centrifugation.
- **Faster Results:** Maximum sensitivity is reached 5–10 minutes after reagent addition.
- **Greater Sensitivity:** The luminescent assay format avoids inherent fluorescent background signals, providing excellent signal-to-background readings. The assay is linear over 3 logs of cell number.

Description:

The Proteasome-Glo™ Cell-Based Assays^(a,b) are homogeneous, luminescent assays that individually measure the chymotrypsin-like, trypsin-like or caspase-like protease activity associated with the proteasome complex in cultured cells. The 26S proteasome is a 2.5MDa multi-protein complex found in all eukaryotic cells. The proteasome is responsible for much of the protein degradation required to maintain cellular homeostasis including degradation of critical cell-cycle proteins, tumor suppressors, transcription factors, inhibitory proteins, and damaged cellular proteins.

The Proteasome-Glo™ Cell-Based Assays provide luminogenic proteasome substrates, in buffers optimized for cell permeabilization, proteasome activity and luciferase activity. Addition of the Proteasome-Glo™ Cell-Based Reagent in an “add-mix-measure” format results in proteasome cleavage of the substrate and rapid generation of a luminescent signal produced by the luciferase reaction.

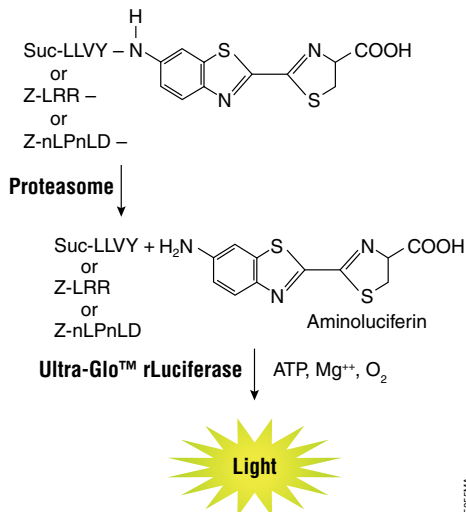


Figure 1: The luminogenic substrate containing the Suc-LLVY, Z-LRR or Z-nLPnLD sequences are recognized by the proteasome. Following proteasome cleavage, the substrate for luciferase (aminoluciferin) is released, allowing the luciferase reaction to occur producing a luminescent signal that is proportional to the amount of proteasome activity present.

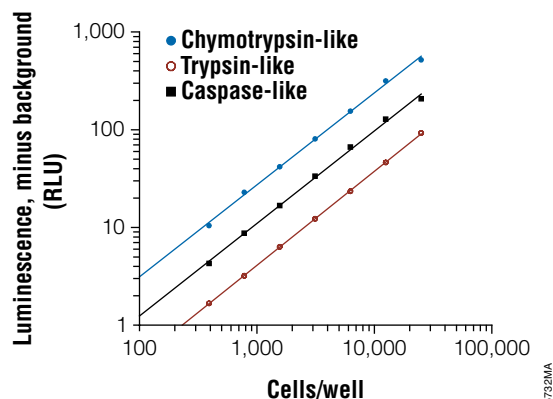


Figure 2. Luminescence is proportional to cell number ($r^2=0.99$, slope 0.94-0.96). A titration of untreated U266 cells was performed in a 96-well plate using the Proteasome-Glo™ Cell-Based Assays. Ten minutes after reagent addition, luminescence was determined at relative light units (RLU) using a DYNEX MLX® plate luminometer. Each point represents the average of four wells. The background (no-cell control) was subtracted from each (average no-cell RLU=3.09-6.54). The r^2 and slope were calculated after transforming the data to a \log_{10} - \log_{10} plot.

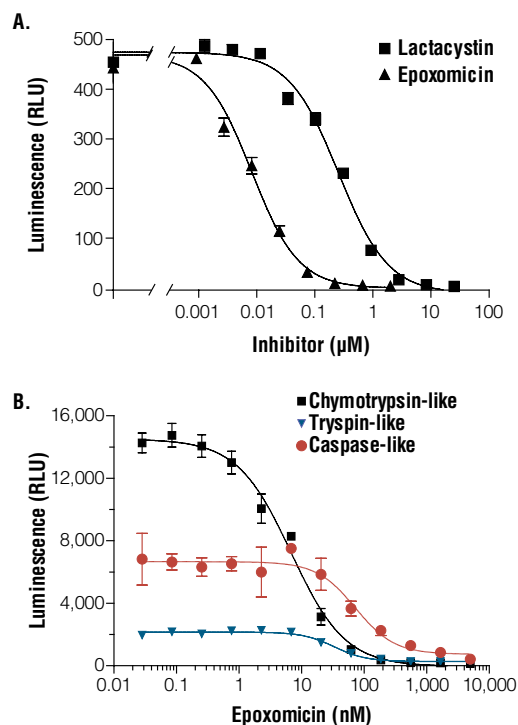


Figure 3. Bioassays using proteasome inhibitors. Panel A. Comparison of lactacystin and epoxomicin using U266 cells (10,000 cells per well in a 96-well plate). Following a 1.75 hour drug treatment chymotrypsin-like activity was measured 15 minutes after addition of Proteasome-Glo™ Reagent using a DYNEX MLX® luminometer. **Panel B.** Epoxomicin inhibition of all sites in a 384-well format. Chymotrypsin-like, trypsin-like and caspase-like activities were individually measured using U266 cells. Five thousand cells per well were treated for 2 hours with various concentrations of epoxomicin. Luminescence was measured 15 minutes after addition of Proteasome-Glo™ Reagents (25µl per well) using a BMG Labtechnologies FLUOstar Optima Luminometer.



Product	Size	Cat.#
Proteasome-Glo™ Chymotrypsin-Like Cell-Based Assay	10ml	G8660
	5 × 10ml	G8661
	2 × 50ml	G8662
Proteasome-Glo™ Trypsin-Like Cell-Based Assay	10ml	G8760
	5 × 10ml	G8761
	2 × 50ml	G8762
Proteasome-Glo™ Caspase-Like Cell-Based Assay	10ml	G8860
	5 × 10ml	G8861
	2 × 50ml	G8862
Proteasome-Glo™ 3-Substrate Cell-Based Assay System	10ml	G1180
	50ml	G1200

Cat. # G8660 is sufficient for 100 assays at 100µl/assay in 96-well plates or 400 assays at 25µl/assay in 384-well plates. Includes:

- 10ml Proteasome-Glo™ Cell-Based Buffer
- 50µl Suc-LLVY-Glo™ Substrate
- 1 bottle Luciferin Detection Reagent

Cat. # G8661 is sufficient for 500 assays at 100µl/assay in 96-well plates or 2,000 assays at 25µl/assay in 384-well plates. Includes:

- 5 × 10ml Proteasome-Glo™ Cell-Based Buffer
- 5 × 50µl Suc-LLVY-Glo™ Substrate
- 5 bottles Luciferin Detection Reagent

Cat. # G8662 is sufficient for 1,000 assays at 100µl/assay in 96-well plates or 4,000 assays at 25µl/assay in 384-well plates. Includes:

- 2 × 50ml Proteasome-Glo™ Cell-Based Buffer
- 2 × 250µl Suc-LLVY-Glo™ Substrate
- 2 bottles Luciferin Detection Reagent

Cat. # G8760 is sufficient for 100 assays at 100µl/assay in 96-well plates or 400 assays at 25µl/assay in 384-well plates. Includes:

- 10ml Proteasome-Glo™ Cell-Based Buffer
- 100µl Z-LRR-Glo™ Substrate
- 15µl Inhibitor 1
- 100µl Inhibitor Mix 2
- 1 bottle Luciferin Detection Reagent

Cat. # G8761 is sufficient for 500 assays at 100µl/assay in 96-well plates or 2,000 assays at 25µl/assay in 384-well plates. Includes:

- 5 × 10ml Proteasome-Glo™ Cell-Based Buffer
- 5 × 100µl Z-LRR-Glo™ Substrate
- 5 × 15µl Inhibitor 1
- 5 × 100µl Inhibitor Mix 2
- 5 bottles Luciferin Detection Reagent

Cat. # G8762 is sufficient for 1,000 assays at 100µl/assay in 96-well plates or 4,000 assays at 25µl/assay in 384-well plates. Includes:

- 2 × 50ml Proteasome-Glo™ Cell-Based Buffer
- 2 × 500µl Z-LRR-Glo™ Substrate
- 5 × 75µl Inhibitor 1
- 2 × 500µl Inhibitor Mix 2
- 2 bottles Luciferin Detection Reagent

Cat. # G8860 is sufficient for 100 assays at 100µl/assay in 96-well plates or 400 assays at 25µl/assay in 384-well plates. Includes:

- 10ml Proteasome-Glo™ Cell-Based Buffer
- 50µl Z-nLPnLD-Glo™ Substrate
- 1 bottle Luciferin Detection Reagent

Cat. # G8861 is sufficient for 500 assays at 100µl/assay in 96-well plates or 2,000 assays at 25µl/assay in 384-well plates. Includes:

- 5 × 10ml Proteasome-Glo™ Cell-Based Buffer
- 5 × 50µl Z-nLPnLD-Glo™ Substrate
- 5 bottles Luciferin Detection Reagent

Cat. # G8862 is sufficient for 1,000 assays at 100µl/assay in 96-well plates or 4,000 assays at 25µl/assay in 384-well plates. Includes:

- 2 × 50ml Proteasome-Glo™ Cell-Based Buffer
- 2 × 250µl Z-nLPnLD-Glo™ Substrate
- 0.2 bottles Luciferin Detection Reagent

Storage Conditions: Store the Proteasome-Glo™ Cell-Based Assay components at -20°C with minimal loss of signal. The Proteasome-Glo™ Chymotrypsin-Like or Caspase-Like Reagent (substrate, buffer and Luciferin Detection Reagent) can be stored at 4°C or -20°C for four weeks with minimal loss of activity. The Proteasome-Glo™ Trypsin-Like Reagent is stable with minimal loss of activity for one week at 4°C or four weeks at -20°C.

Related Products

Product	Size	Cat.#
Proteasome-Glo™ Glo 3-Substrate System	10ml	G8531
Proteasome-Glo™ Glo Chymotrypsin-Like Assays	10ml	G8621
Proteasome-Glo™ Glo Trypsin-Like Assays	10ml	G8631
Proteasome-Glo™ Glo Caspase-Like Assays	10ml	G8641
Calpain-Glo™ Protease Assay	10ml	G8501
	50ml	G8502
DPPIV-Glo™ Protease Assay	10ml	G8350
	50ml	G8351
CellTiter-Glo® Luminescent Cell Viability Assay (ATP)	10ml	G7570
CellTiter-Blue® Cell Viability Assay (Resazurin)	20ml	G8080
CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS)*	200 assays	G3582
CytoTox 96® Non-Radioactive Cytotoxicity Assay (LDH)	1,000 assays	G1780
CytoTox-ONE™ Homogeneous Membrane Integrity Assay	1,000 assays	G7892
Caspase-Glo® 8 Assay*	100ml	G8202
Caspase-Glo® 9 Assay*	100ml	G8212
Caspase-Glo® 3/7 Assay*	100ml	G8092
	10 × 100ml	G8093
Caspase-Glo® 2 Assay	100ml	G0940
	50ml	G0941
Caspase-Glo® 6 Assay	10ml	G0970
	50ml	G0971
Apo-ONE® Homogeneous Caspase-3/7 Assay	100ml	G7791
MAO-Glo™ Assay	200 assays	V1401
	1,000 assays	V1402
P450-Glo™ CYP1A1 Assay	50ml1	V8752
P450-Glo™ CYP1B1 Assay	50ml1	V8762
P450-Glo™ CYP1A2 Assay	50ml1	V8772
P450-Glo™ CYP2C8 Assay	50ml1	V8782
P450-Glo™ CYP2C9 Assay	50ml1	V8792
P450-Glo™ CYP3A4 Assay	50ml1	V8802
P450-Glo™ CYP3A7 Assay	50ml1	V8812
P450-Glo™ CYP2D6 Assay	50ml1	V8892
P450-Glo™ CYP2C19 Assay	50ml1	V8882
P450-Glo™ CYP1A2 Screening System	1,000 assays	V9770
P450-Glo™ CYP2C9 Screening System	1,000 assays	V9790
P450-Glo™ CYP3A4 Screening System	1,000 assays	V9800
P450-Glo™ CYP2D6 Screening System	1,000 assays	V9890
P450-Glo™ CYP2C19 Screening System	1,000 assays	V9880
PgP-Glo™ Assay System	10ml	V3591
PgP-Glo™ Assay System with P-glycoprotein	10ml	V3601

*For Laboratory Use.

Additional Sizes Available.

(a) U.S. Pat. No. 6,602,677, Australian Pat. No. 754312 and other patents pending.

(b) The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.

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