

Thermo Scientific Proteasome Redistribution[®] Assay

The Redistribution technology monitors the cellular translocation of GFP-tagged proteins in response to drug compounds or other stimuli and allows easy acquisition of multiple readouts from the same cell in a single assay run. In addition to the primary readout, high content assays provide supplementary information about cell morphology, compound fluorescence, and cellular toxicity.

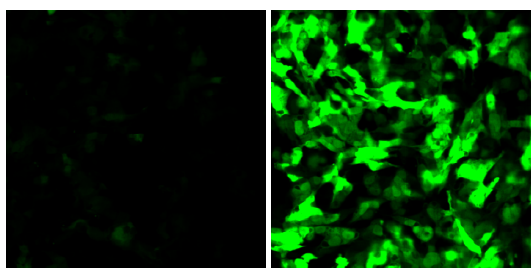


Figure 1. Inhibition of proteasomal degradation of Ubi(G76V)-EGFP by MG-132. Cells were treated with 5 μ M MG-132 for 4 hr (right panel) or vehicle control (left panel). The increase in fluorescence intensity is detected by the image analysis algorithm.

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The ubiquitin/proteasome pathway is the main non-lysosomal route for intracellular protein degradation in eukaryotes and is implicated in the degradation of proteins that control vital processes such as cell cycle progression, signal transduction, differentiation, and apoptosis. Covalent attachment of multiple ubiquitin molecules to proteins targets them for proteolytic degradation by a complex cellular structure, the 26S proteasome [1]. A cascade of reactions catalyzed by ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3) is required to attach the ubiquitin moiety to a lysine residue of the protein targeted for degradation. Ubiquitination is a reversible process due to a number of de-ubiquitinating enzymes that mediate the disassembly of ubiquitin-protein conjugates. Proteasomal degradation, on the other hand, is irreversible and is therefore appropriate for unidirectional control of cellular pathways. The 26S proteasome is a multicatalytic multisubunit protease where the core proteasome (20S)

consists of two heptameric outer α -rings and two inner β -rings responsible for the proteolytic activity of the proteasome. The 19S regulatory complex is stacked on the ends of the cylindrical core to form a 26S proteasome and is involved in an ATP-dependent recognition and unfolding of proteins targeted for degradation. Some deubiquitinating enzymes are associated with the 26S proteasome, making them key regulators of the proteasome-dependent protein degradation process.

Features

- Designed to assay compounds for their ability to modulate proteasomal activity
- Coupled to EGFP for easy monitoring of the cellular translocation event
- Robust cell-based assay for use in high content analysis and fluorescence microscope applications

Highlights:

- **Biologically relevant data**
Compounds tested in a cellular environment
- **Validated**
Functionally tested cells provided with an optimized assay protocol
- **Easy to use**
Just plate cells, add compounds, and image

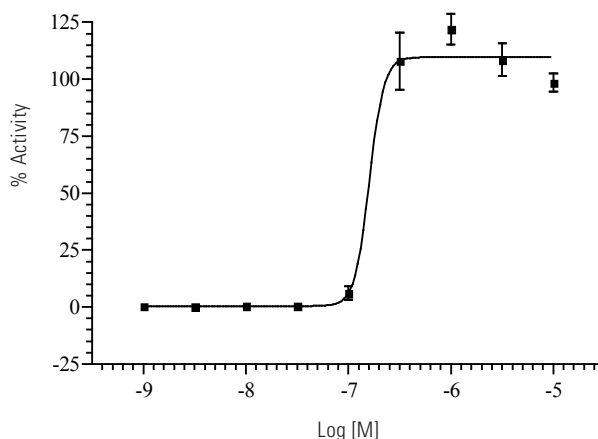
MG-132 concentration response curve
in the Proteasome Redistribution assay

Figure 2. Concentration response curve in the Proteasome assay: MG-132 concentration response (n=12); the EC_{50} is approximately 0.2 μ M. Concentration response was measured in 9 point half log dilution series. Cells were treated with test compound for 4 hr. Cells were then fixed and increase in fluorescence intensity was measured using the Cellomics ArrayScan V^{TI} Reader and the Redistribution V3 BioApplication. % activity was calculated relative to the positive (5 μ M MG-132) and negative control (0.25% DMSO).

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Assay Details

Recombinant U2OS cells stably expressing human Ubiquitin (aa 1-82) carrying a Gly76Val mutation, Ubi(G76V) fused to the N-terminus of enhanced green fluorescent protein (EGFP). The Proteasome assay is designed to assay for proteasome inhibitors by monitoring ubiquitin/proteasome-dependent proteolysis of a GFP-based substrate. The GFP-substrate consists of a mutated uncleavable ubiquitin moiety (Ubi[G76V]) fused to GFP resulting in constitutive degradation of the protein [2]. Degradation of Ubi[G76V]-EGFP can be inhibited by the irreversible proteasome inhibitors MG-132 [3] and Lactacystin [4]. In this assay MG-132 is used as reference compound. Compounds are assayed for their ability to inhibit proteasomal activity. Compounds causing a decrease in Ubi(G76V)-EGFP degradation are directly interfering with the 26S proteasome. The Proteasome assay is validated with an average $Z' = 0.75 \pm 0.05$, suitable for both screening and profiling applications.

Imaging

The Proteasome Redistribution assay can be imaged on most HCS platforms and fluorescence microscopes. The filters should be set for Hoechst (350/461 nm) and GFP/FITC (488/509 nm) (wavelength for excitation and emission maxima). Consult the instrument manual for

the correct filter settings. The translocation can typically be analyzed on images taken with a 10x objective or higher magnification. The primary output in the Proteasome Redistribution assay is accumulation of Ubi(G76V)-EGFP in the nucleus. The data analysis should therefore report an output relating to the GFP fluorescence intensity in the nucleus.

Imaging on Thermo Scientific Cellomics ArrayScan V^{TI}

This assay has been validated on the Cellomics Arrayscan V^{TI} using a 10x objective (0.63X coupler), XF100 filter sets for Hoechst and FITC, and the Redistribution V3 BioApplication. The output used was MEAN_CircAvgInten. The minimally acceptable number of cells used for image analysis in each well was set to 100 cells. Other BioApplications that can be used for this assay include Molecular TranslocationV2, CompartmentalAnalysisV2, NucTransV2, and ColocalizationV3.

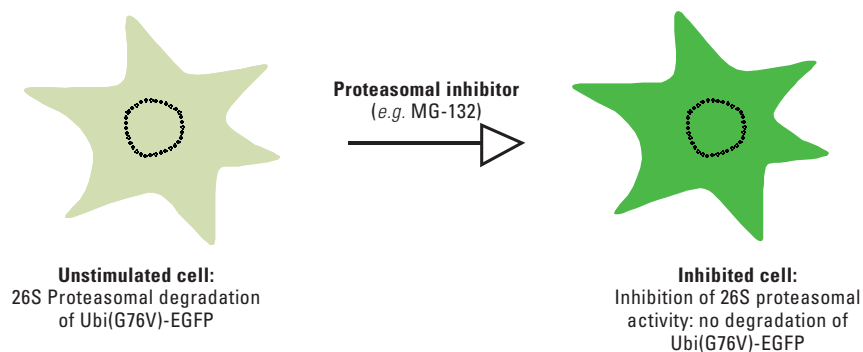


Figure 3. Illustration of the Proteasome Redistribution assay.

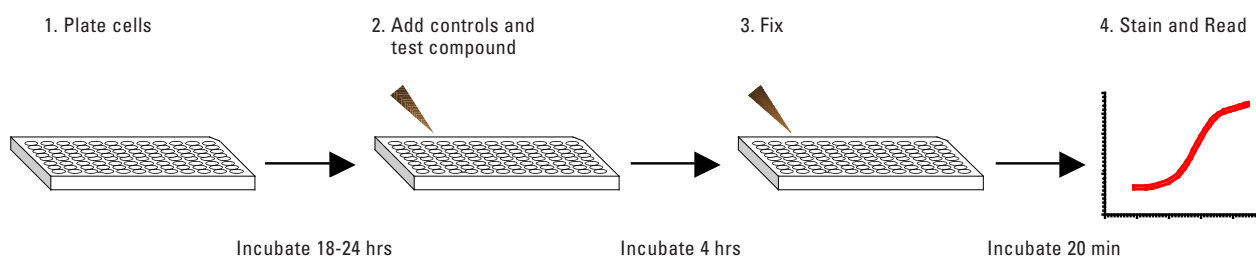


Figure 4. The Proteasome Redistribution assay is very easy and fast to perform.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
021_01	Proteasome Redistribution Assay	U2OS	•	•	•

The Redistribution Assays are available in 3 product formats, Profiling, Screening and CryoRedi, for different volume and level of convenience needs. The Redistribution Assays can also be accessed through the Thermo Scientific Managed Services.

Related Thermo Scientific Products

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
052_01	SCF-Skp2 E3 ligase: p27 degradation Redistribution Assay	HeLa	•	•	
055_01	E6-AP E3 ligase: p53 degradation Redistribution Assay	HeLa	•	•	
8407601	Cellomics LC3B Detection HCS Reagent Kit	Antibody- and dye-based reagent kit			
8408001	Cellomics Multiparameter Cell Death Detection HCS Reagent Kit	Antibody- and dye-based reagent kit			
8407701	Cellomics Poly-Ubiquitin Detection HCS Reagent Kit	Antibody- and dye-based reagent kit			
CX03004-INS	Cellomics ONE BioApplication Suite	High content data acquisition and analysis software			
CX03102A/B	Cellomics ArrayScan V ^{TI}	Flexible, high throughput, high content reader			
N01-3001	CellWoRx	Economical high content reader			

References

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2. Dantuma NP et al., *Nature*, 18, 538-543, 2000.
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4. Lee DH & Goldberg A, *J. Biol. Chem.* 271, 27280-27284, 1996.

