PROTEOSTAT®
Aggresome detection kit

Robust, quantitative detection of aggresomes relevant to the study of neurodegenerative disease, liver disease and toxicology

Aggresomes are inclusion bodies of aggregated, misfolded proteins that form when the ubiquitin-proteasome protein-degradation machinery is overwhelmed. Typically, aggresomes form in response to cellular stress and provide a cytoprotective mechanism by isolating misfolded proteins, leading ultimately to their clearance by autophagy. Protein aggregate formation is furthermore a hallmark of a variety of human diseases, such as Alzheimer's, Parkinson's, Amyotrophic lateral sclerosis or alcoholic liver disease. The PROTEOSTAT® Aggresome Detection Reagent contained in this kit is a molecular rotor dye. While in solution, free intramolecular rotation along a single central bond prevents fluorescence. The PROTEOSTAT® dye specifically intercalates into the cross-beta spine of quaternary protein structures typically found in misfolded and aggregated proteins, which will inhibit the dye's rotation and lead to a strong fluorescence. PROTEOSTAT® Aggresome Detection kit provides a rapid, specific and quantitative approach to label aggresomes in cells, eliminating the need of artificial protein mutations. PROTEOSTAT® dye has been validated under a wide range of conditions and with small molecule modulators, demonstrating its suitability for compound screening. Furthermore, it is suitable for multiplex co-immunofluorescence to study your target of interest in the context of autophagy and aggresome formation.

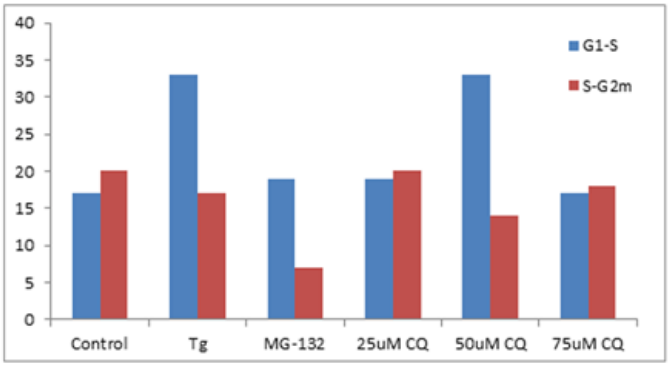
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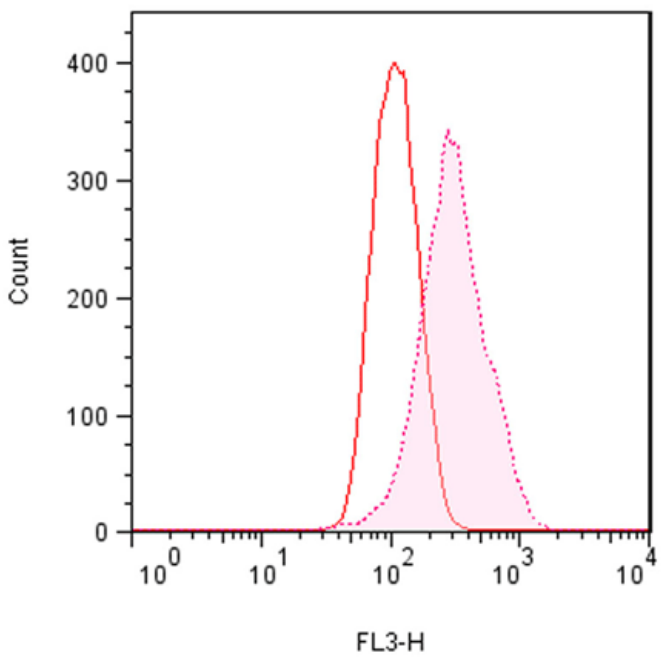
ENZ-51035-0025	25 tests
ENZ-51035-K100	100 tests

Manuals, SDS & CofA View Online »

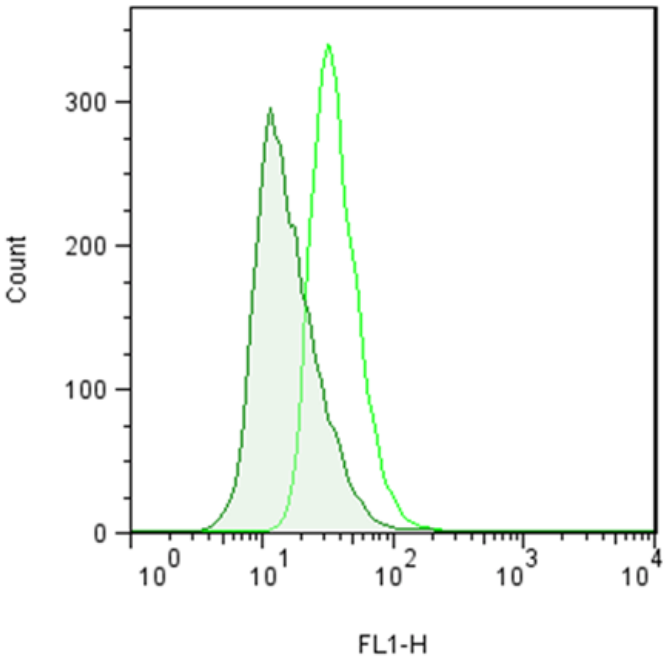
- Provides a sensitive cell-based assay of drug responsiveness to identify inhibitors relevant to neurodegenerative disease in an authentic cellular context
- Reliable and simple assay doesn't require non-physiological protein mutations or genetically engineered cell lines
- Validated under a wide range of conditions and with small molecule modulators demonstrating suitability for screening compounds of potential therapeutic value
- Fixed-cell assay is optimized for antibody co-localization studies
- Easily quantifies aggresome and related inclusion bodies by flow cytometry
- Useful for the study of neurodegenerative diseases, liver disease, toxicology studies and much more



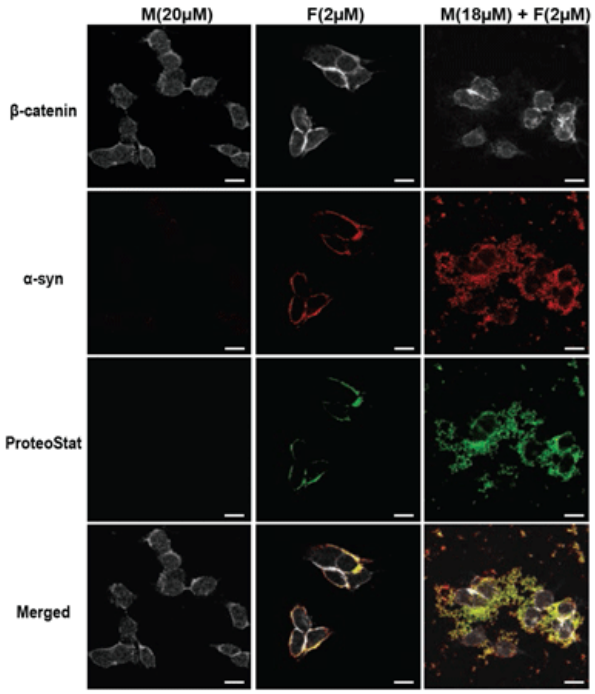
K562 cells were treated with reagents to induce ER stress and incomplete autophagy using Thapsigargin (Tg, 0.1 µM), 25, 50 and 75 µM Chloroquine (CQ) for 24 h respectively. A positive control was employed using the proteasome inhibitor, MG-132 (5 µM) for 24 h. Pelleted cells were fixed and permeabilized. Cells were then labelled for 30 min at RT with 300 µl PROTEOSTAT® Aggresome Detection reagent (Enzo Life Sciences) according to the manufacturer's instructions and 1 µg/ml DAPI (for cell cycle determination). Relative increase in Aggresome Propensity Factor (APF) of S phase above that of G1 and that of G2m above S phase was determined for each treatment and compared to control values. ER stress inducer, Tg and 50 µM CQ was shown to both up-regulate Aggresomes more in S phase than G1 compared to controls. While proteasome inhibitor, MG-132 and 50 µM CQ was shown to down-regulate Aggresomes more in G2m than S phase than control levels.Courtesy of the Flow Cytometry Core Facility, Blizard Institute, Queen Mary University of London, London, UK.



Flow cytometry-based analysis. Jurkat cells were mock-induced with 0.2% DMSO or induced with 5 µM MG-132 overnight at 37°C. After treatment, cells were fixed and incubated with PROTEOSTAT® dye, then analyzed by flow cytometry without washing using a 488 nm laser in the FL3 channel. In MG-132 treated cells, fluorescent red signal increases about 3-fold. The described assay allows assessment of the effects of protein aggregation.



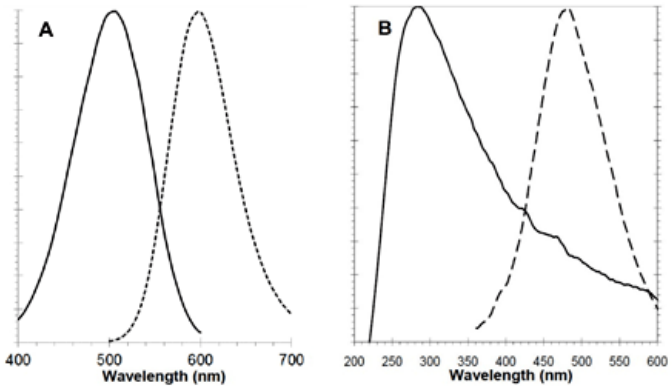
Flow cytometry-based analysis. Jurkat cells were mock-induced with 0.2% DMSO or induced with 5 µM MG-132 overnight at 37°C. After treatment, cells were fixed and incubated with Fluorescein-p62 Ab (1:500 dilution of stock), then after washing, analyzed by flow cytometry using a 488 nm laser in the FL1 channel. In MG-132 treated cells, Fluorescein-p62 Ab signal increases about 2.5-fold.



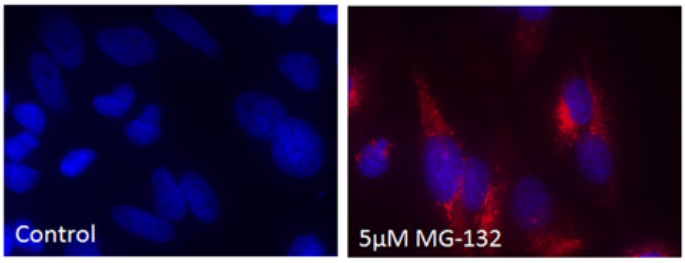
α-syn mixture forms aggregates at the cell plasma membrane that are internalized into the M17 neuroblastoma cell line. M17 cells were treated with α-syn monomers (M), sonicated α-syn PFFs (F) or α-syn mixture (M+F). After 4 days, M17 cells were washed 3 times prior to fixation and then stained with the PROTEOSTAT® aggregation dye (green) and immunostained against α-syn (red) and β-catenin (grey). Scale bars = 20 µm. Image used with permission from authors from “Fibril growth and seeding capacity play key roles in α-synuclein-mediated apoptotic cell death. A-L Mahul-Mellier, et al.; Cell Death & Differentiation (2015).” (doi:10.1038/cdd.2015.79).



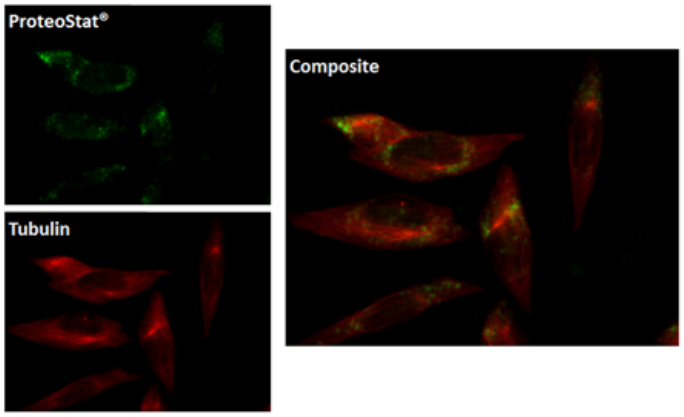
ProteoStat® Aggresome Detection Kit



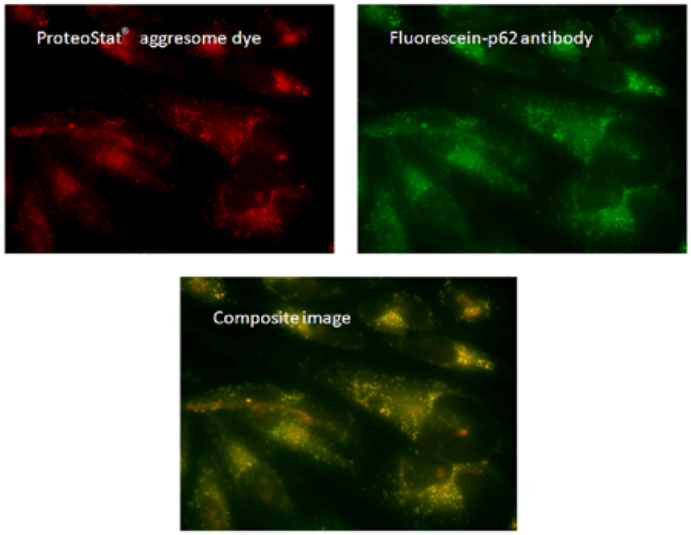
Emission/Excitation Spectra of PROTEOSTAT® Dye and Nuclear Stain



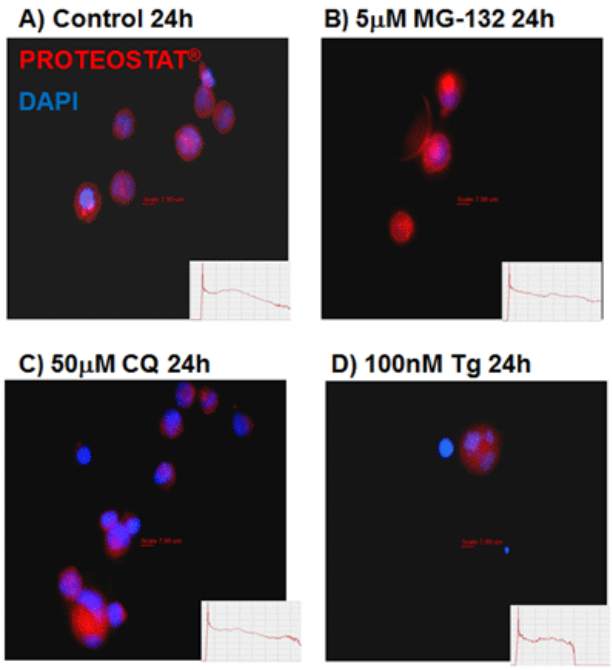
Aggresomes with HeLa cells, treated for 12 hours with 5µM MG-132 (right), detected by ProteoStat® Aggresome dye (red) and counterstained with Hoechst 33342. Control panel on left is untreated.



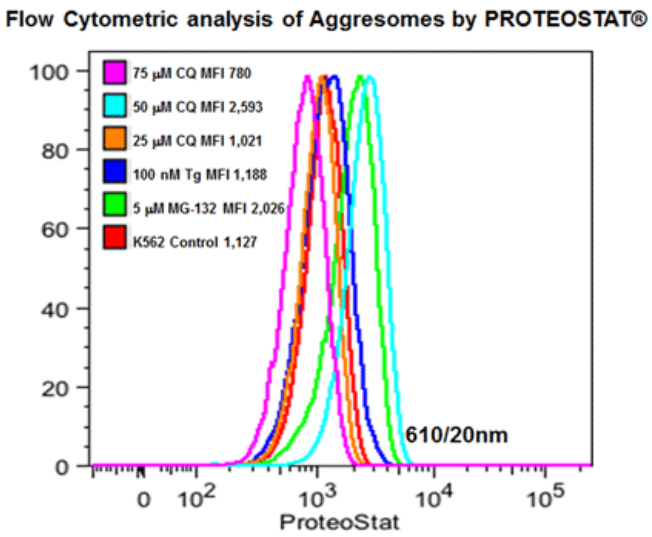
Aggresomes with HeLa cells, previously treated for 12 hours with 5µM MG-132, detected by ProteoStat® Aggresome dye (upper left, appearing here in green) showing co-localization with Alexa Fluor® 647-Tubulin antibody (lower left, appearing in red) and composite image (right), as observed by fluorescence microscopy.



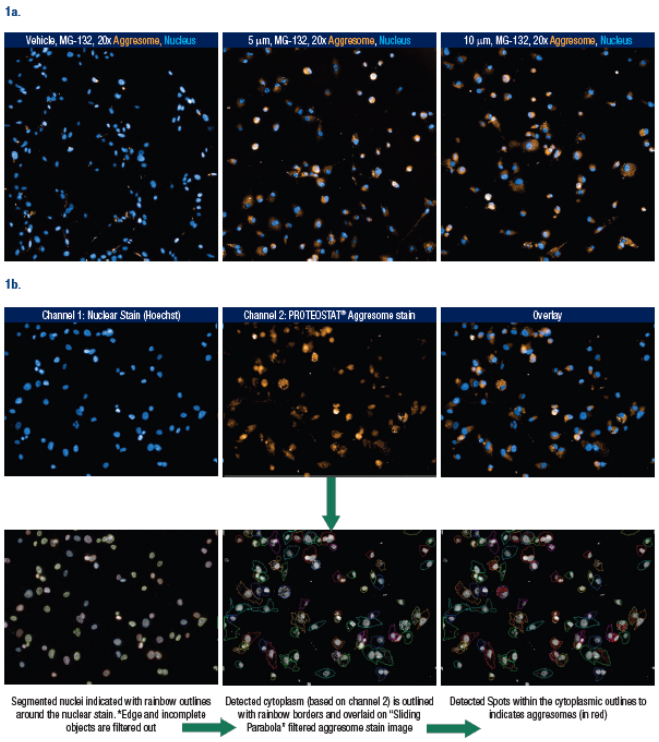
Aggresomes within HeLa cells, previously treated for 12 hours with 5µM MG-132, detected by ProteoStat® aggresome dye (upper left) showing co-localization with fluorescein-p62 antibody (upper right) and composite image (lower center), as observed by fluorescence microscopy.



Aggresomes and aggresome-like inclusion bodies (ALIS) produced by ER stress related autophagy were imaged by epi-fluorescence microscopy. K562 cells were A) untreated or treated with B) 5 mM MG-132 C) 50 mM CQ or D) 100 nM thapsigargin (Tg) for 24 h. Cells were then fixed and permeabilised and incubated with PROTEOSTAT® dye (1:10,000 dilution) for 30 min at RT. PROTEOSTAT® staining indicating aggresomes & ALIS are shown in red with DAPI staining shown in blue. Courtesy of the Flow Cytometry Core Facility, Blizard Institute, Queen Mary University of London, London, UK.



Flow cytometric analysis of protein aggresomes induced by ER stress related autophagy. K562 cells were untreated or treated with 25, 50, 75 mM CQ, 100 nM thapsigargin (Tg) or 5 mM MG-132 for 24 h. Cells were then fixed and permeabilised and incubated with PROTEOSTAT® dye (1:10,000 dilution) for 30 min at RT. Cells (30,000) were then analysed on the blue 610/20nm channel of a BD LSRII. Courtesy of the Flow Cytometry Core Facility, Blizard Institute, Queen Mary University of London, London, UK.



Aggresome detection in U87MG cells.
a) Fluorescent images of vehicle vs 5µM and 10µM MG-132 treated (18 hours) U87MG cells.
b) Representation of image segmentation performed by Operetta CLS™ to determine quantification in 1a

Handling & Storage

Use/Stability	With proper storage, the kit components are stable for one year from date of receipt.
Handling	Protect from light. Avoid freeze/thaw cycles.
Short Term Storage	-20°C
Long Term Storage	-80°C
Shipping	Blue Ice

Regulatory Status

RUO - Research Use Only

Product Details

Application	Flow Cytometry, Fluorescence microscopy, Fluorescent detection
Application Notes	The PROTEOSTAT® Aggresome detection kit assay is potentially applicable to the identification of small molecules that inhibit aggresome formation as well as immuno-localization studies between aggregated protein cargo and the various proteins implicated in aggresome formation, such as histone deacetylase 6, parkin, ataxin-3, dynein motor complex and ubiquilin-1.
Contents	PROTEOSTAT®Aggresome detection reagent Hoechst 33342 Nuclear stain Proteasome Inhibitor (MG-132) 10X Assay Buffer
Quality Control	A sample from each lot of PROTEOSTAT® Aggresome detection kit is used to stain Jurkat cells and analyzed by flow cytometry, using the procedures described in the user manual. The aggresome activity factor (AAF) values for the samples are greater than 25.
Quantity	For ENZ-51035-0025: 25 flow cytometry assays or 50 microscopy assays For ENZ-51035-K100: 100 flow cytometry assays or 200 microscopy assays

Technical Info / Product Notes

The PROTEOSTAT® Aggresome Detection Kit is a member of the CELLESTIAL® product line, reagents and assay kits comprising fluorescent molecular probes that have been extensively benchmarked for live cell analysis applications. CELLESTIAL® reagents and kits are optimal for use in demanding imaging applications, such as confocal microscopy, flow cytometry and HCS, where consistency and reproducibility are required.

Featured In:

High-throughput image-based aggresome quantification (SLAS Discov 2020; 25:783-791).

Application: Optimized to monitor aggresome formation in a miniaturized, automated, and quantitative phenotypic assay. The approach is validated by screening a chemical library of 1280 compounds.

Application Note:

A Red-emitting Fluorescent Probe for Rapid Detection of Protein and Peptide Aggregates in Post-mortem Human Brain Tissue Sections from Patients Diagnosed with Alzheimer's and Parkinson's Disease

Monitoring the Accumulation and Clearance of Exogenously Introduced Beta-amyloid in a Cell-Based Model of Alzheimer's Disease by Fluorescence Microscopy and Fluorescence Microplate Assay

Towards Understanding the Molecular Basis of Parkinson's Disease: Cell-based Model of Mitophagy and Aggresome Accumulation

Detection of bacterial aggregation by flow cytometry

Cell-Based Screening of Focused Bioactive Compound Libraries: Assessing Small Molecule Modulators of the Canonical Wnt Signaling and Autophagy-Lysosome Pathways

Monitoring Whole Brain Plaque Burden with PROTEOSTAT® Aggresome Detection Kit and Light Sheet Microscopy

Cited samples:

For an overview on cited samples please click here.

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