

ProteoStat® Aggresome Detection Kit

for flow cytometry and fluorescence microscopy

Aggresomes are inclusion bodies that form when the ubiquitin-proteasome machinery is overwhelmed with aggregation-prone proteins [H. Amijee, et al.; Biochem. Society **37**, 692 (2009)]. Typically, an aggresome forms in response to some cellular stress, such as hyperthermia, viral infection or exposure to reactive oxygen species. Aggresomes appear to provide a cytoprotective function by sequestering the toxic, aggregated proteins and may also facilitate their ultimate elimination from cells by autophagy. Certain cellular inclusion bodies associated with human disease are thought to arise from an aggresomal response, including Alzheimer's disease as it relates to intracellular and extracellular aggregates, Lewy bodies associated with neurons in Parkinson's disease, Mallory bodies associated with liver cells in alcoholic liver disease and hyaline inclusion bodies associated with astrocytes in amyotrophic lateral sclerosis [A. Dedeoglu, et al.; Journal of Neuroscience **22**, 8942, (2002)].

ProteoStat® Aggresome detection kit contains a novel 488 nm excitable red fluorescent molecular rotor dye to specifically detect denatured protein cargo within aggresomes and aggresome-like inclusion bodies in fixed and permeabilized cells. The detection reagent supplied in the ProteoStat® Aggresome detection kit becomes brightly fluorescent upon binding to aggregated proteins within vacuoles produced during aggresome formation and has been validated under a wide range of conditions in which the autophagy and proteasome pathways are known to be modulated. MG-132 (Prod. No. BML-PI102), a proteasome inhibitor, is included as a positive control in the kit. A nuclear counter-stain is provided in the kit as well to highlight this organelle.

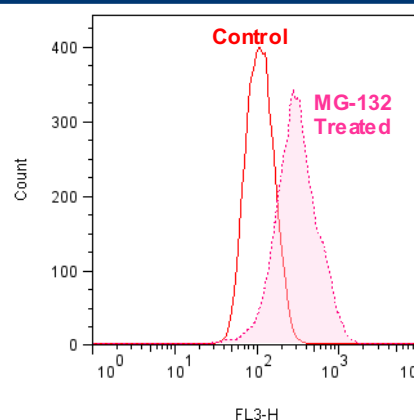
A Novel Cell-based Assay for the Detection of Aggresomes *NEW*

ProteoStat® Aggresome Detection Kit

ENZ-51035-K100

100 Assays

- 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 that doesn't require non-physiological protein mutations or genetically engineered cell lines
- Fixed-cell assay is optimized for antibody colocalization studies to identify interactions between aggregated protein cargo and various proteins implicated in aggresome formation
- Validated under a wide range of conditions and with small molecule modulators demonstrating suitability for screening compounds of potential therapeutic value
- Easily quantifies aggresome accumulation by flow cytometry



| Treatment | Mean (FL3) Signal |
|------------------------|-------------------|
| Control | 113 |
| Treatment (5µM MG-132) | 335 |

FIGURE 1: Identifying aggresomes by flow cytometry. Jurkat cells were mock-induced with 0.2% DMSO or induced with 5µM MG-132 overnight at 37°C. After treatment, cells were fixed and incubated with ProteoStat® dye, then analyzed by flow cytometry. Results are presented as histogram overlays. In MG-132 treated cells, fluorescent red signal increases about 3-fold.

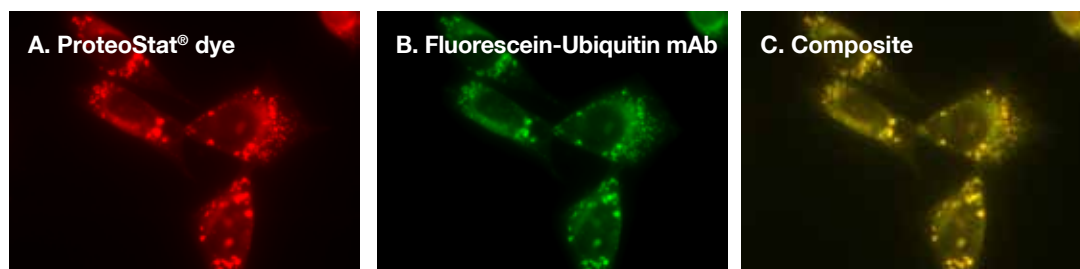


FIGURE 2: ProteoStat™ dye co-localizes with Fluorescein-labeled Ubiquitin mAb

Cell aggresome detected by ProteoStat® dye co-localizes with fluorescein-Ub mAb (clone EX-9), as observed by fluorescence microscopy: HeLa cells were treated for 12 hours with 5µM MG-132 on a glass slide (a) ProteoStat® dye stained cells. (b) Fluorescein-Ub mAb-labeled cells (c) Composite image.

Ordering Information

| Product | Prod. No. | Size |
|--|----------------|------------|
| ProteoStat® Aggresome detection kit | ENZ-51035-K100 | 100 Assays |

Related Products

| Product | Prod. No. | Size |
|--|------------------|---------------------|
| Cyto-ID™ Autophagy detection kit | ENZ-51031-K200 | 200 Assays |
| Proteasome 26S degradation kit | BML-PW-8950-0001 | 96 Assays |
| Ubiquitin activating kit | BML-UW0400-0001 | 96 Assays |
| Ubiquitin conjugating kit (HeLa lysate-based) | BML-UW9915-0001 | 20 x 50µl reactions |
| Ubiquitinylation kit | BML-UW9920-0001 | 50 x 50µl reactions |

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