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Measurement of protein aggregation with PROTEOSTAT

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Protocol status: Working

We use this protocol and it's working

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Abstract

Measurement of protein aggregation with PROTEOSTAT

Fluorescence plate-based detection

- 1 Prepare the PROTEOSTAT detection dye according to the manufacturer's instructions.
- 2 Dilute the detection dye 1:2 in 1× assay buffer (ENZ-51023-KP050).
- 3 Load 2 µL of diluted detection dye into each well of a 96-well plate with black walls and a clear bottom.
- 4 For each sample, load 30 µg of total protein and dilute in assay buffer (if needed) to a final volume of 100 µL.
- 5 Incubate the plate for 15 minutes at room temperature in the dark.
- 6 Measure fluorescence at 550 nm excitation and 600 nm emission using a fluorescence plate reader (PerkinElmer Envision 2105).

Imaging-based detection

- 7 Use the PROTEOSTAT Aggresome Detection Kit (Cat. No. ENZ-51035-KIT) for imaging-based detection.
- 8 Fix cells or tissue sections and permeabilize with 0.1% Triton X-100 in PBS.
- 9 Incubate samples with detection reagent for 30 minutes at room temperature in the dark, according to the manufacturer's instructions.
- 10 Wash samples with PBS.
- 11 Counterstain samples with DAPI.
- 12 Image using a Leica TCS SP8 confocal microscope with a 60×/1.4 NA oil immersion lens.

