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Measurement of protein aggregation with PROTEOSTAT

In 1 collection

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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.