# PROTEOSTAT® Protein aggregation assay

# Quantitative Detection of Protein Aggregates from Visible to Subvisible

**PROTEOST**AT<sup>®</sup> Protein aggregation assay provides a simple, homogenous assay format for monitoring peptide and protein aggregation in solution. This is useful for defining optimal storage formulations for proteins, for screening of compounds that promote or inhibit protein aggregation and potentially for the sensitive measurement of molecular chaperone activity. The assay can be employed to streamline protein processing and formulation optimization procedures. Relative to conventional protein aggregation detection dyes, such as Thioflavin T, the Enzo PROTEOSTAT® detection reagent can detect aggregates from a broader range of proteins, yields a much brighter signal, provides at least 2 orders of magnitude linear dynamic range and offers superior performance across a broad range of pH values (4~10) and buffer compositions. Sensitivity for this assay is in the sub-micromolar range and as little as 1-5% protein aggregate is detectable in a concentrated protein solution. The assay is capable of providing quantitative analysis of protein aggregation in a robust and high-throughput fashion (Z' factor score >0.5). Lyophilized native and aggregated protein are provided as negative and positive controls for monitoring changes in protein aggregation status.

PROTEOSTAT<sup>®</sup> Protein Aggregation Standards kit (Prod. No. <u>ENZ-51039</u>) is the *only commercially available* protein aggregation standards assay with stabilized, high-quality reference samples for generating trace protein aggregate levels in concentrated monomeric IgG. Easy to use - *simply add water!* 

Citations: 85

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# **Ordering Information**

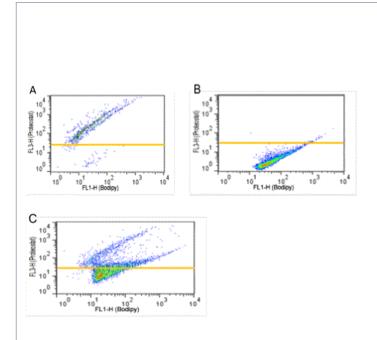
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ENZ-51023-KP050	50 tests
ENZ-51023-KP002	2x96 tests

- A simple, sensitive, homogenous fluorescent assay
- Validated for use with microplate or flow cytometry platform
- Extensively benchmarked with IgG
- Optimize buffers and excipients for protein formulation
- Performs with a wide pH and ionic strength range
- Use with <u>PROTEOSTAT® Protein</u> <u>Aggregation Standards</u> for accurate quantification of aggregated protein in solution.

Manuals, SDS & CofA

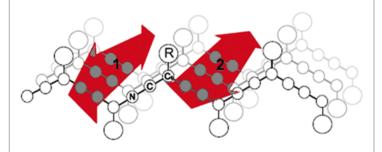
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9000 8000 PROTEOSTAT® Detection Reagent y=36179x 7000 R2=0.997 Thioflavin T 6000 y=603.47x R2=0.756 5000 RFU 4000 3000 2000 1000 10% 12% % of Aggregate

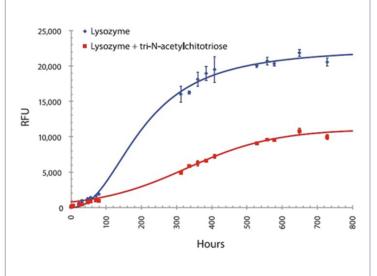
Figure 2. Flow Cytometry Application: PROTEOSTAT<sup>®</sup>
Dye Mixed with Bodipy Dye (Pyrromethene 546)
Differentiates Oil Droplets From True Protein
Aggregates. Bodipy vs PROTEOSTAT<sup>®</sup> fluorescence of IgG aggregates (A), Silicon oil droplets (B) and a mixture of Silicon oil droplets and IgG aggregates (C).

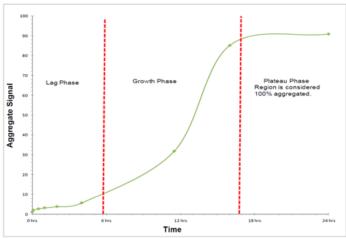
Effective linear dynamic range for antibody aggregate detection using PROTEOSTAT® Detection Reagent compared with Thioflavin T. Relative fluorescence unit values (RFUs) may differ depending upon the microplate reader employed for the analysis.

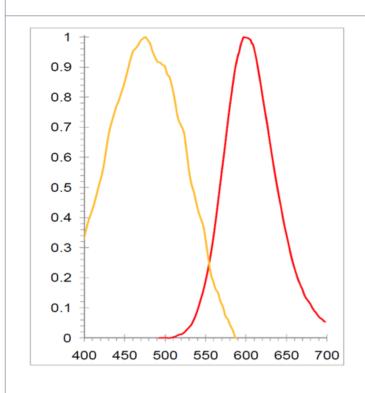


Dye is immobilized when bound to the aggregate and begins to fluoresce.

Thioflavin T: early prototype dye in the design of PROTEOSTAT<sup>®</sup> assay which also rotates around a single bond (red arrow) in the absence of protein aggregates.







## **Handling & Storage**

**Use/Stability** With proper storage, the kit components are stable up to the date noted on the product

label. Store kit at -20°C in a non-frost free freezer or -80°C for longer term storage.

Short Term Storage -20°C

Long Term Storage -80°C

Shipping Dry Ice

# Regulatory Status RUO - Research Use Only

### **Product Details**

**Application** Flow Cytometry, Fluorescence microscopy, Fluorescent

detection, Microplate

Application Notes This kit has been specifically designed for monitoring of

protein aggregate formation in solution.

**Contents** PROTEOSTAT<sup>®</sup> detection reagent

PROTEOSTAT<sup>®</sup> positive control PROTEOSTAT<sup>®</sup> negative control 10X PROTEOSTAT<sup>®</sup> assay buffer

**Quality Control**A sample of PROTEOSTAT<sup>®</sup> Protein aggregation assay

was used to assay (1) 20  $\mu$ M aggregated lysozyme, (2) 20  $\mu$ M native lysozyme; and (3) mixtures of 5% aggregate. The Z' factor is greater than 0.5 and the 5% aggregate signal is greater than 3 standard deviations above the no-

aggregate control.

Quantity For ENZ-51023-KP050: 50 tests in a 96 well plate or 16

flow cytometry tests

For ENZ-51023-KP002: 2 x 96-well tests or 70 flow

cytometry tests

#### Technical Info / Product Notes

#### **Application Notes:**

Particle analysis of therapeutic protein formulations with ImageStreamX® Imaging Flow Cytometry and the PROTEOSTAT® Protein Aggregation Assay

Prediction of Aggregation Propensity and Monitoring of Aggregation of Antibody-Drug Conjugates (ADC) using ProteoStat<sup>®</sup> Reagents

Enzo and PROTEOSTAT are trademarks of Enzo Life Sciences, Inc. Several of Enzo's products and product applications are covered by US and foreign patents and patents pending.

#### Cited samples:

PROTEOSTAT® Cited Samples

Last modified: May 29, 2024



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