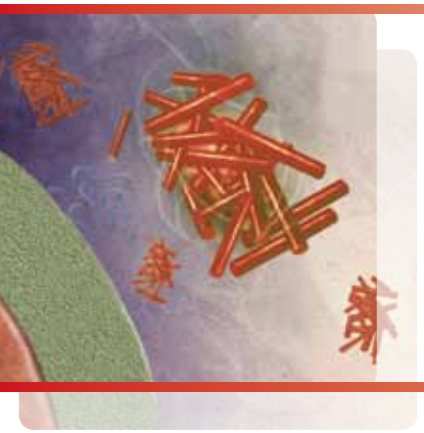

ProteoStat™ Protein Aggregation Assay

Biochemical assays for monitoring protein aggregates often rely upon ultracentrifugation, size-exclusion chromatography, gel electrophoresis, dynamic light scattering, or turbidity measurements. These techniques are not capable of working for every protein, nor are the assays ideal for tackling the wide range of aggregation problems that can arise during the manufacture of protein pharmaceuticals.

ProteoStat™ Protein aggregation assay provides a simple, homogenous assay format for monitoring peptide and protein aggregation. The assay can be employed to streamline protein processing and optimize formulation procedures during from protein expression through manufacturing. Relative to conventional protein aggregation detection dyes, such as Thioflavin T, the ProteoStat™ detection reagent can detect a broader range of different protein aggregates. The assay 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 of this assay is in the sub-micromolar range so that less than 1% protein aggregate is detectable in a protein solution. The assay delivers Z' factor scores greater than 0.5 providing quantitative analysis of protein aggregation in a robust and high-throughput fashion. Lyophilized native and aggregated proteins are included in the kit as negative and positive controls for monitoring changes in protein aggregation status.

ProteoStat™ Protein Aggregation Assay *NEW*

ENZ-51023-KP002 2 x 96 wells

Aggregation is one of the most significant obstacles to the development of protein-based pharmaceuticals. During drug formulation, protein aggregation can lead to low yield, poor storage capacity and increased production costs.

The ProteoStat™ Protein aggregation assay is suitable for detecting protein aggregation caused by different environments to which a protein product might be exposed, such as pH, temperature, light, oxygen, mechanical stress and freeze/thaw cycles.

- A simple, sensitive, homogenous fluorescent micro-plate assay
- Extensively benchmarked with IgG
- Optimize buffers and excipients for protein formulation
- Performs with a wide pH and ionic strength range

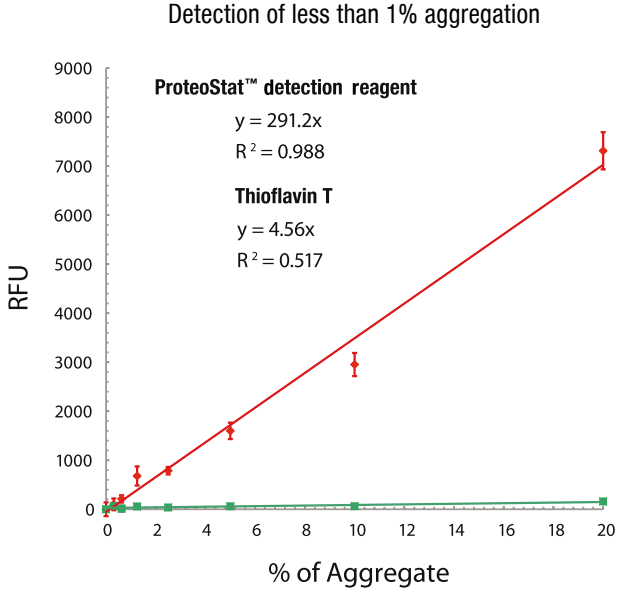

FIGURE 1: Effective linear dynamic range for antibody aggregate detection using the Enzo Life Sciences ProteoStat™ detection reagent compared with Thioflavin T: Rabbit anti-goat IgG (4.26mg/ml) was incubated in aqueous HCl solution, pH 2.7 at 80° for 90 minutes to form aggregates. The signal from the aggregate was determined after mixing aggregate with monomer at different ratios

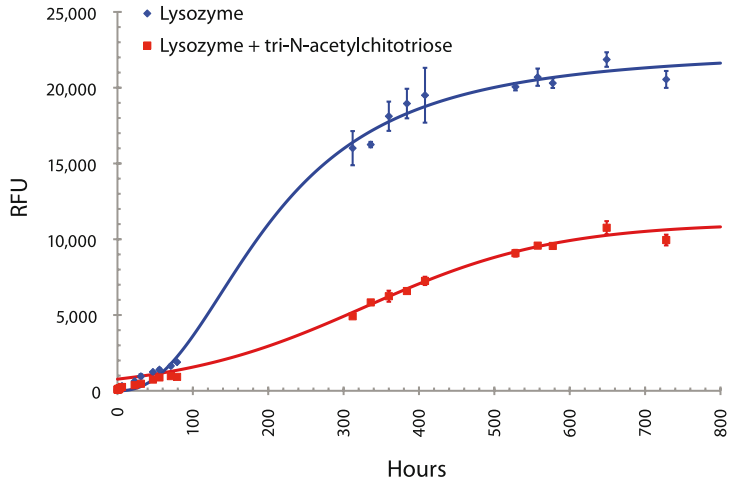

FIGURE 2: Monitoring the ability of an excipient to inhibit protein aggregation: Hen egg white lysozyme (300µM) was incubated in the presence or absence of N,N',N"-triacetyl-chitotriose, a lysozyme aggregation inhibitor, (chitotriose, 600µM) in 10mM potassium phosphate, pH 7.3 for 16 hours. Aggregation was induced by 3.5 fold dilution into 50mM potassium phosphate buffer, pH 12.2. Aggregation was followed by removing an aliquot of the protein and diluting such that the final composition was 20µM protein, 50mM potassium phosphate buffer, pH 7 and 3µM ProteoStat™ detection reagent . Protein was incubated with the dye for 15 minutes prior to determining the fluorescence using a BioTek Synergy Mx microplate reader, with an excitation setting of 515nm and emission setting of 603nm. The zero time point was taken before dilution into pH 12.2 buffer. Triplicate measurements were made from samples deposited in a Greiner µClear® black, clear bottom 96-well microplate. Aggregation was monitored for several weeks at room temperature. The Enzo Life Sciences dye readily demonstrates that Chitotriose inhibits lysozyme aggregation.

Assay Mechanism

Proteins misfold and aggregate under stress from pH levels, temperature, buffers, light, oxygen, mechanical agitation and freeze/thaw cycles. Applications performed with the ProteoStat™ Protein aggregation dye can quantify aggregates in protein samples.

ProteoStat™ is a molecular rotor dye that rotates like a propeller in the absence of protein aggregates and does not fluoresce. When the dye binds to the aggregate, it is immobilized – slowing down the rotational movement and causing the dye to fluoresce. Unlike other environmentally sensitive dyes that measure unfolding and exposure of hydrophobic regions of proteins, there is little interference from hydrophobic compounds or detergents present at normal concentrations.

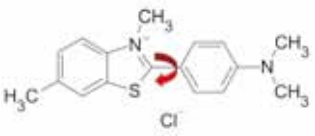

FIGURE 3: Thioflavin T: early prototype dye in the design of ProteoStat™ assay which also rotates around a single bond (red arrow) in the absence of protein aggregates.

FIGURE 4: Dye is immobilized when bound to the aggregate and begins to fluoresce.

Ordering Information

Product	Prod. No.	Size
ProteoStat™ Protein aggregation assay	ENZ-51023-KP002	2 x 96 wells

Related Products

Product	Prod. No.	Size
ProteoStat™ Thermal shift stability assay	ENZ-51027-K400	400 Assays
Protein A EIA kit	ADI-900-057	1 x 96 wells

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