PROTEOSTAT® Protein Aggregation Standards

Catalog #: ENZ-51039-KP002
2 x 96-well plate assays

**NOTE:** This version contains a change to shipping condition of product.
For the latest product information, including support documentation, visit us online:
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DESCRIPTION

The PROTEOSTAT® Protein Aggregation standards are ideal for reliable and accurate quantification of protein aggregation in solution and has been developed for use with the PROTEOSTAT® Protein Aggregation assay (ENZ-51023-KP050 and ENZ-51023-KP002). The standards are used to determine low levels of aggregated protein in a sample by comparing the assay response of a test sample to that of the standard curve; comprised of standards with known concentrations of aggregated IgG. Protein test samples and protein aggregation standards should be analyzed in the same manner, by mixing them with detection reagent and using a fluorescence microplate reader to measure the fluorescence intensity values. As with any protein assay, different protein aggregates will elicit slightly greater or lesser fluorescence intensity response based upon their inherent amino acid composition and sequence. It is recommended that a standard curve be prepared each time the PROTEOSTAT® Protein Aggregation assay (ENZ-51023-KP050 or ENZ-51023-KP002) is performed.

The PROTEOSTAT® Protein Aggregation standards contain stabilized, high-quality reference samples for generating an accurate standard curve. The standard curve is formulated from different amounts of aggregated IgG, combined with monomeric IgG. Once reconstituted, the samples contain 12.5%, 6.25%, 3.13%, 1.56%, 0.78%, 0.39%, 0.20% and 0% aggregated protein, while the total protein concentration of each standard is maintained at 1mg/mL. The total protein concentration of the IgG standard has been calibrated by direct comparison to an internal protein standard, in order to ensure lot-to-lot consistency and accuracy.

Orthogonal characterization methods, including laser-based nanoparticle tracking and micro-flow imaging, have been employed to validate monomer integrity during manufacturing.
QUALITY CONTROL
A sample from each lot of PROTEOSTAT® Protein Aggregation standards is used with the procedures described in the user manual. Perform assay with 12.5% - 0% Aggregate in duplicate. Linear curve R-Square has to be greater than 0.95.

MATERIALS PROVIDED

Components:
A. PROTEOSTAT® Control (0% aggregate), 1 vial
B. PROTEOSTAT® Control (0.20% aggregate), 1 vial
C. PROTEOSTAT® Control (0.39% aggregate), 1 vial
D. PROTEOSTAT® Control (0.78% aggregate), 1 vial
E. PROTEOSTAT® Control (1.56% aggregate), 1 vial
F. PROTEOSTAT® Control (3.13% aggregate), 1 vial
G. PROTEOSTAT® Control (6.25% aggregate), 1 vial
H. PROTEOSTAT® Control (12.5% aggregate), 1 vial
I. Deionized Water, 5mL

Other:
User Manual

OTHER MATERIALS NEEDED
• Fluorescence microplate reader with a filter set or monochromator
• Setting of Excitation = ~550 nm/Emission = ~600 nm.
• 96-well microplate: We recommend a black wall microplate with a clear bottom.
• Calibrated, adjustable precision pipetters, preferably with disposable plastic tips.
• PROTEOSTAT® Protein aggregation assay kit (ENZ-51023-KP050 or ENZ-51023-KP002).

STABILITY AND STORAGE CONDITIONS:
All reagents are shipped on blue ice (-20°C). Upon receipt, the kit should be stored upright and protected from light at ≤-20°C in a non-frost free freezer. When stored properly, these reagents are stable for at least twelve months. Avoid repeated freezing and thawing.
SAFETY WARNINGS & PRECAUTIONS

- This product is for research use only. Not for diagnostic procedures.
- Some components of this kit may contain hazardous substances. Reagents can be harmful if ingested or absorbed through the skin and may cause irritation to the eyes. Reagents should be treated as possible mutagens and should be handled with care and disposed of properly.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas. All blood components and biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.
- To avoid photobleaching, perform all manipulations in low light environments or protected from light by other means.

ASSAY PROCEDURE

1) All standards (8 tubes) are supplied as stabilized, lyophilized powders. Allow all reagents to thaw at room temperature. Briefly centrifuge all the tubes for 5 sec at 16,000 RPM and add 440 μL deionized water to each tube to generate 1mg/mL stock solutions.

2) Gently mix the protein standards on a rotating, rocking mixer, such as a Nutator® (TCS Scientific, New Hope, PA), for 30 min. and briefly centrifuge samples again before performing the aggregate quantification experiment.  
*Note: Do not vortex or cause unnecessary bubbles as this may lead to aggregation which could affect results. Do not centrifuge at high speed or for long time, as the aggregates are in suspension, not in solution.*

3) Prepare the 96-well microplate with 98 μL of test protein per well. Typically, standard curves are constructed using two replicates for each point on the curve for each plate. Unused stock standard samples may be stored in aliquots at 4°C for several days.
4) Obtain PROTEOSTAT® Detection Reagent from PROTEOSTAT® Protein Aggregation Assay kit (ENZ-51023-KP050 or ENZ-51023-KP002). Dilute PROTEOSTAT® Detection Reagent according to manual of ENZ-51023 and dispense 2 μL of the diluted PROTEOSTAT® Detection Reagent into each well.

5) Incubate the microplate containing test samples and standards in the dark for 15 minutes at room temperature.

6) Read the generated signal with a fluorescence microplate reader, using an excitation setting of ~550 nm and an emission setting of ~600 nm. NOTE: Please refer to the instructions provided in the ENZ-51023 manual for detailed instructions.

![Figure 1](image1.png)

**Figure 1:** Typical standard curve produced using the PROTEOSTAT® Protein Aggregation standards.

![Figure 2](image2.png)

**Figure 2:** The total protein concentration of the IgG standards have been calibrated by direct comparison to an internal bovine gamma globulin (BGG) protein standard in order to ensure lot-to-lot consistency and accuracy.