

# PROTEOSTAT®

## Thermal shift stability assay kit

### Accelerated screening for protein stability as a function of pH, ionic strength, and concentration.

The PROTEOSTAT® Thermal shift stability assay kit includes a fluorescent dye which detects protein aggregation, so it can be used to monitor protein stability under systematic thermal stress conditions. From the thermal shift assay, a temperature at which the bulk of the protein becomes aggregated can readily be identified. The aggregation temperature is an indicator of protein stability and can be used to optimize conditions that minimize protein aggregation as well as to identify ligands that bind and confer structural stability to a protein of interest. Use the related PROTEOSTAT® Protein Refolding and Aggregation Sensing Kit (Prod. No. ENZ-51040) to identify optimal protein refolding conditions. Conditions that increase the aggregation temperature, increase the stability of the protein.

Citations: 17

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### Ordering Information

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ENZ-51027-K100	100 tests
ENZ-51027-K400	400 tests

### Manuals, SDS & CofA

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- A simple, sensitive, homogenous fluorescence assay with no need for sample separation or dilution
- Able to optimize protein stability conditions in an accelerated manner
- Can detect ligand binding, even without a prior knowledge of the protein's function or ligand binding site
- Works over a wide temperature, pH and ionic strength range and compatible with commonly used buffers and excipients
- Provides a convenient, complementary orthogonal method for cross-validation of instrumentation-intensive techniques

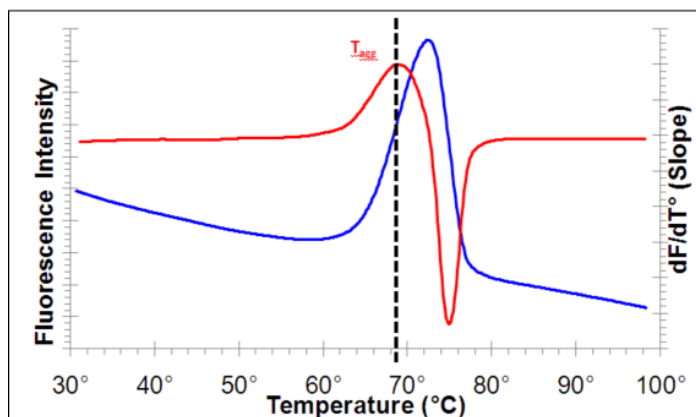


Figure 1. Typical results of the PROTEOSTAT<sup>®</sup> Thermal Shift Stability Assay are shown for goat anti-mouse IgG (11.2 mg/ml at pH 7.4). Using a RT-PCR instrument programmed to ramp the temperature from 30° to 99°C at a 3°/ minute rate, while reading the fluorescence continuously. The blue line represents the raw fluorescence data, and the red line shows the corresponding first derivative trace, highlighting the slope of the fluorescence intensity curve. The first derivative plot provides the aggregation temperature of the protein (Tagg: The point of maximal slope).

### ProteoStat<sup>™</sup> Dye's $T_{agg}$ Value is not Equivalent to SYPRO<sup>®</sup> Orange Dye's $T_m$ Value

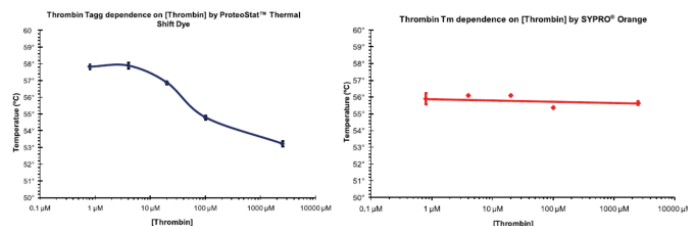


Figure 2. PROTEOSTAT<sup>®</sup> Dye's Aggregation Temperature is not equivalent to the Melting Temperature Obtained Using SYPRO<sup>®</sup> Orange. SYPRO Orange dye is an environment-sensitive dye that binds to the hydrophobic regions of unfolded proteins. It can be used to measure  $T_m$ .  $T_m$  is the temperature mid-point of a thermal unfolding curve. At the temperature midpoint  $T_m$ , one half of the target proteins in a sample are unfolded, and one half of the target proteins in the sample remain folded. Unfolding does not depend upon neighboring proteins. ProteoStat is a molecular rotor dye that binds to the surface of protein aggregates. Tagg refers to the mid-point of a thermal aggregation curve. Proteins unfold and then subsequently aggregate. Aggregation depends upon neighboring proteins. The higher the protein concentration, the greater the tendency to aggregate.

# 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.
Handling	Protect from light. Avoid freeze/thaw cycles.
Short Term Storage	-20°C
Long Term Storage	-20°C
Shipping	Blue Ice

## Regulatory Status

RUO - Research Use Only

# Product Details

Application Notes	This kit has been designed for monitoring protein stability under systematic thermal stress conditions.
Contents	PROTEOSTAT® TS Detection Reagent β-Lactoglobulin control 10X Assay Buffer
Quality Control	A sample kit from each lot of PROTEOSTAT® Thermal shift stability assay kit is tested using the procedure described in the manual. The detected aggregation temperature for 16µg/ml of β-lactoglobulin is a single peak at 76° ± 2°C.

**Application Note:**

[Prediction of Aggregation Propensity and Monitoring of Aggregation of Antibody-Drug Conjugates \(ADC\) using ProteoStat® Reagents](#)

**Cited samples:**

[PROTEOSTAT® Cited Samples](#)

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