

# ProteoStat™ Thermal Shift Stability Assay

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ENZ-51027-K400

400 Assays

**HIGHLIGHT**

- High-throughput thermal shift assay for determining the impact of ligand-protein interactions on target protein stability
- Suitable for accelerated analysis of parameters impacting monoclonal antibody and recombinant protein stability (pH, ionic strength, cryoprotectants, excipients)
- Measures aggregation temperature, providing more flexibility than assays using environment-sensitive dyes to measure unfolding temperature, where detergent and hydrophobic compound interference can be problematic
- Assay is readily performed using a wide range of thermally-controlled fluorescence reading devices (Fluorimeters or real-time PCR instruments)
- Stringently manufactured, to control and eliminate non-specific assay artifacts

Identification of potentially adverse effects of drug candidates on a target protein are typically investigated during the development of lead candidates. The ProteoStat™ assay provides an improved thermal shift approach for assessment of protein stability through monitoring protein aggregation, rather than protein unfolding; minimizing problems encountered with environment-sensitive dyes arising from the use of detergents, membrane proteins and hydrophobic compounds. The ProteoStat™ dye is a proprietary 488 nm excitable molecular rotor probe that under standard aqueous conditions is minimally fluorescent, but upon binding to the surface of aggregated proteins emits a strong red signal at ~600 nm, thus providing a homogeneous assay for analysis of protein stability.

Forced degradation studies involve exposing a protein to harsher conditions than the pharmaceutical product would normally experience and determining at what point it degrades. ProteoStat™ Thermal shift stability assay provides a rapid high-

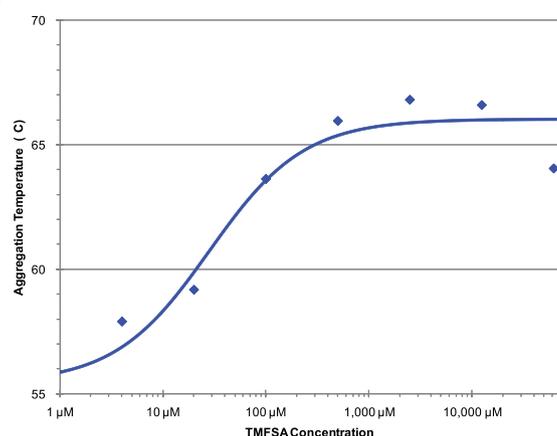
throughput fluorescence-based approach for assessing a range of parameters impacting the stability of monoclonal antibodies and recombinant therapeutic proteins, based upon their exposure to systematically increasing thermal stress, under a variety of solution conditions, in forced degradation studies. This procedure is based upon the expectation that proteins degraded in this manner reflect the degradation pathway(s) experienced during a product's lifetime. Overall protein stability is assessed through determination of the temperature required to induce protein aggregation, as monitored by the ProteoStat™ dye. The assay can be performed using either a conventional fluorimeter equipped with heating control or a real-time PCR instrument. The assay facilitates analysis of protein stability over a wide range of pH values and ionic strengths, as well as using a variety of buffer, cryoprotectant and excipient compositions. The described assay facilitates understanding of the underlying mechanisms impacting protein stability, as well as predicting the stability of therapeutic proteins upon long-term storage, screening the

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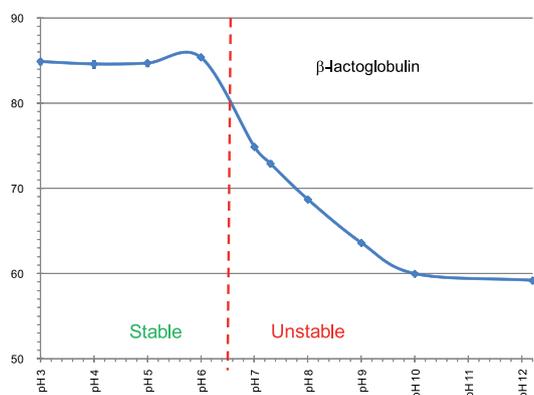
relative stability of protein variants (e.g. amino acid substitution/deletion mutants, PEGylated proteins) and providing other critical information pertaining to the development of optimal protein formulations.

The ProteoStat™ Thermal shift stability assay kit includes a fluorescent dye which detects protein aggregation arising from thermally-induced protein structural destabilization. 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 enhance protein stability as well as to identify ligands that bind and confer structural stability to a protein of interest. Conditions that increase the aggregation temperature, increase the stability of the protein. The assay facilitates understanding of the underlying mechanisms impacting protein stability, and because it is not dependent upon measuring exposed hydrophobic regions arising from protein unfolding is more tolerant of detergents, micelle formation and certain ligands and proteins possessing hydrophobic characteristics.



**FIGURE 1:** Ligand stabilization of protein structure: Carbonic anhydrase I (1 µM) was incubated with 0 to 62.5mM of TMFSA (trifluoromethane sulfonamide) in 25mM MES, 50mM NaCl, pH 6.1. The ProteoStat™ Thermal shift stability assay demonstrates that ligand binding increases protein thermal stability by an amount proportional to the concentration of the ligand.



**FIGURE 2:** Stability of a protein in different buffers: β-Lactoglobulin was diluted into 50mM buffer at different pHs in the presence of 150mM NaCl, and the aggregation temperature was determined using ProteoStat™ dye.

## Related Products

| Product                                      | Prod. No.       | Size         |
|--|-----------------|--------------|
| <b>ProteoStat™ Protein aggregation assay</b> | ENZ-51023-KP002 | 2 x 96 wells |

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