

## Thermal Shift Assay

A valuable tool providing insights into thermal stability and ligand binding properties of proteins, aiding in the characterization & optimization of protein-based research and drug discovery projects.

Thermal shift assay, or differential scanning fluorimetry/protein thermal shift assay, is widely used in structural biology and drug discovery to study the thermal stability and ligand binding properties of proteins.

Thermal shift assay is based on the principle that the thermal denaturation of proteins leads to exposure of hydrophobic regions, which can interact with hydrophobic dyes or fluorescent probes. By monitoring changes in fluorescence intensity, it is possible to determine the protein's melting temperature ( $T_m$ ), which is a measure of stability.

### Applications:

- Determination of protein stability.
- Evaluation of ligand binding.
- Comparative studies of protein variants or mutants.
- Optimization of protein purification and formulation conditions.
- Analysis of protein-protein interactions.

### Experimental Design

Required materials: 0.2 mL of each protein sample at a concentration of 1 mg/mL or greater.



Protein-dye mixture is subjected to a gradual increase in temperature using a thermocycler or a real-time PCR instrument. Temperature is increased at a constant rate of 1-2 degrees Celsius per minute.

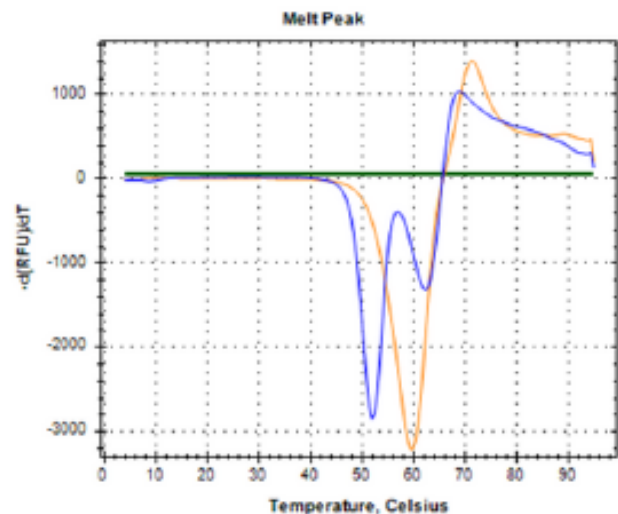


Fluorescence intensity continuously monitored during thermal ramp.



Obtained fluorescence versus temperature data is analyzed to determine the  $T_m$  of the protein.

### Protein Variant Composition



Variant	T <sub>m</sub> 1 (°C)	T <sub>m</sub> 2 (°C)	Comments
#1-blue	52.0	62.5	Two thermal transitions
#2-orange	60.0	---	Single thermal transition

