

Thermal Shift Assay

The thermal shift assay is a valuable tool that provides insights into the thermal stability and ligand binding properties of proteins, aiding in the characterization and optimization of protein-based research and drug discovery projects.

Thermal shift assay, also known as differential scanning fluorimetry or protein thermal shift assay, is a widely used technique in the field of structural biology and drug discovery. It is employed to study the thermal stability and ligand binding properties of proteins.

The thermal shift assay is based on the principle that the thermal denaturation of proteins leads to the 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 its 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. The temperature is increased at a constant rate of 1-2 degrees Celcius per minute.

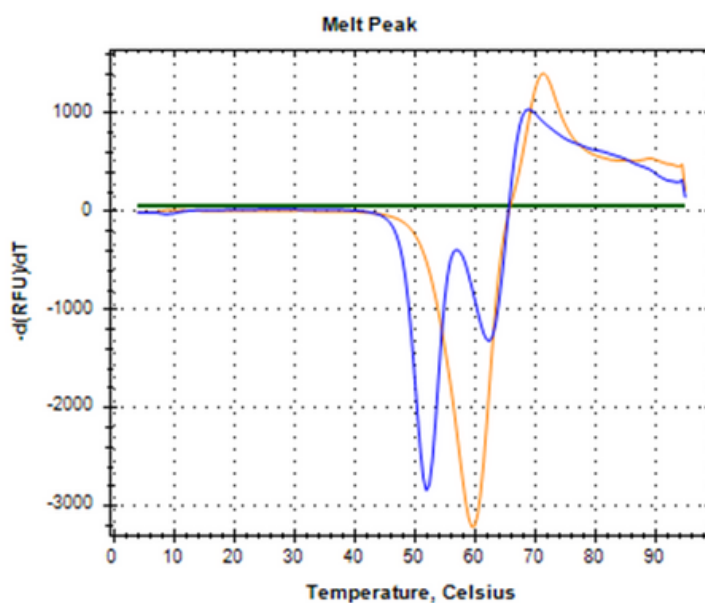


Fluorescence intensity is continuously monitored during the thermal ramp.



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

Protein variant comparison



Variant	T_m 1 (°C)	T_m 2 (°C)	Comments
#1-blue	52.0	62.5	Two thermal transitions
#2-orange	60.0	---	Single thermal transition