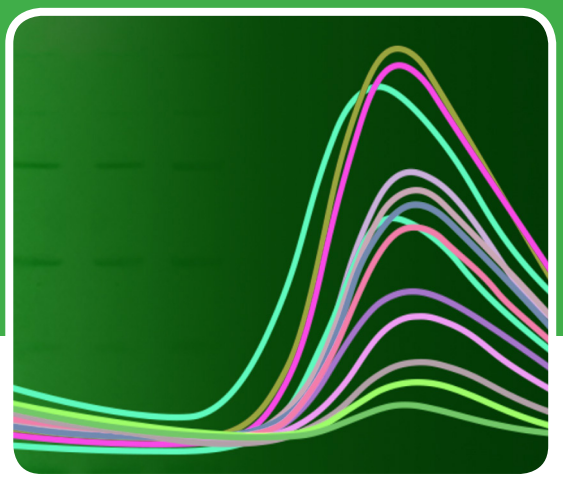


Protein Thermal Stability

GloMelt™ kits for thermal shift assay

GloMelt™ dye undergoes fluorescence enhancement upon binding to hydrophobic regions of denatured proteins. Therefore the dye can be used to detect protein unfolding or measure thermal stability by performing a thermal shift assay, also called Protein Thermal Shift™, differential scanning fluorimetry, or Thermofluor assay.

The thermal shift assay is a rapid and inexpensive technique that quantifies change in protein denaturation temperature, and thus can be used to screen conditions that affect protein thermal stability, such as protein mutations, ligand binding, and buffer formulations (like pH, salts, detergents, and other additives). These assays are rapid (typically about 30 minutes) and are performed on a quantitative PCR system. The thermal shift method is compatible with high throughput screening and requires much less protein than methods such as circular dichroism and differential scanning calorimetry.

GloMelt™ Advantages

- Optimized for detection in qPCR instruments
- Compatible with wide pH range, reducing agents, and common buffers/excipients
- Tolerant to high detergent concentrations
- Ideal for high throughput assays, low reaction volumes and low protein concentrations
- Improved reproducibility when used with ROX reference dye
- GloMelt™ dye is highly soluble and stable in aqueous buffers

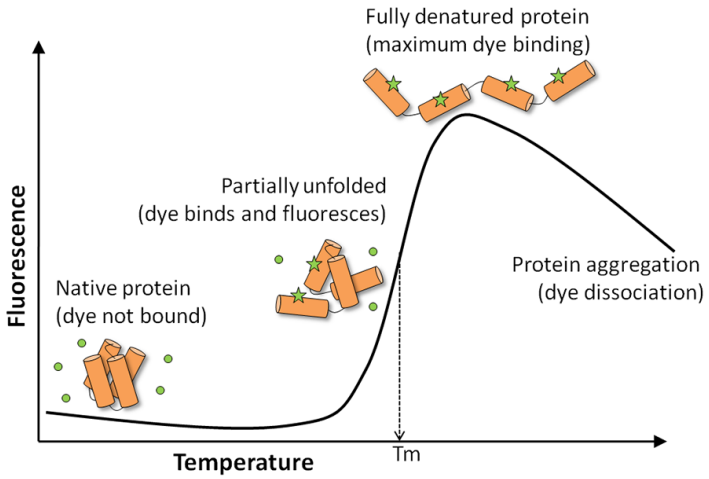


Figure 1. Environmentally sensitive fluorescent dyes can be used to monitor the temperature dependent unfolding of a protein. The protein's melting temperature (Tm) is a reporter of the protein's thermal stability.

Applications

- Optimize buffer formulation for protein stability and storage
- Determine how mutations affect your protein's stability
- Rapidly screen small molecule drug candidates and other ligands for protein binding

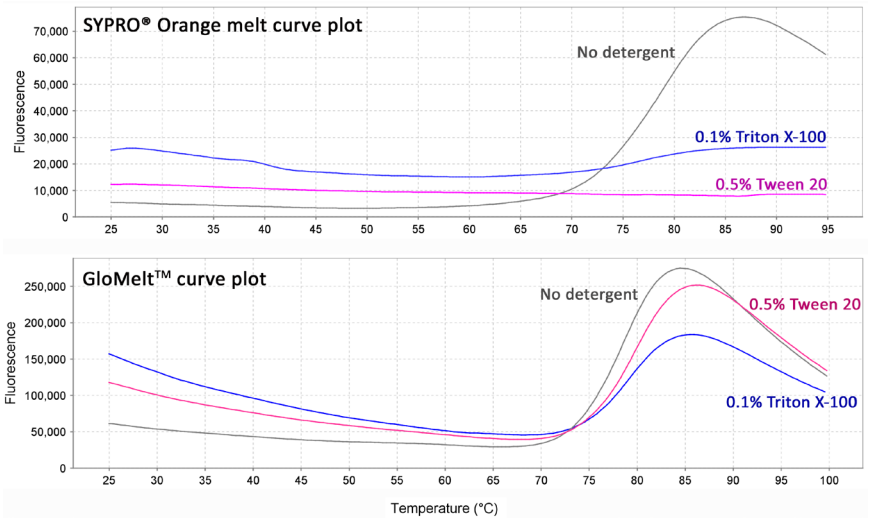
Ordering Information

Cat. #	Product name	Unit size
33021-T	GloMelt™ Thermal Shift Protein Stability Kit	200 x 20 uL reactions
33021-1		2000 x 20 uL reactions
33022-T	GloMelt™ Thermal Shift Protein Stability Kit (with ROX)	200 x 20 uL reactions
33022-1		2000 x 20 uL reactions

Biotium

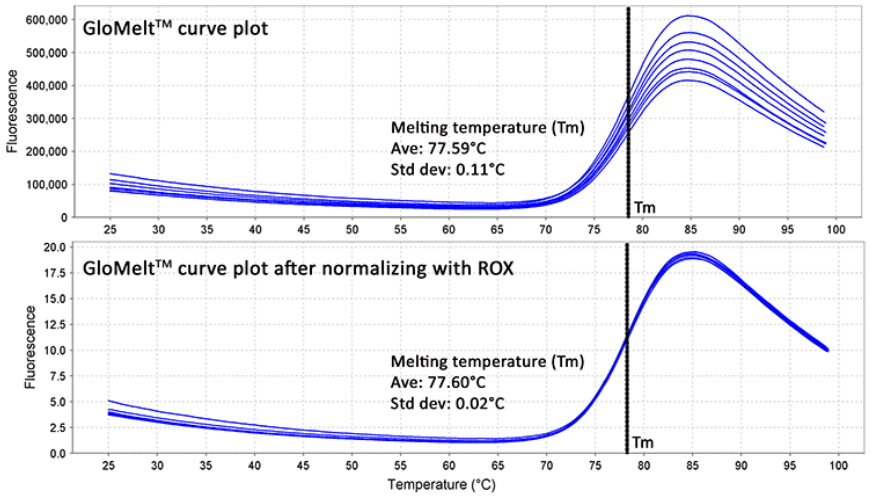
GloMelt™ dye has significant advantages over other environmentally sensitive dyes, such as SYPRO® Orange and PROTEOSTAT® TS dye:

- GloMelt™ dye generates a strong signal because it is optimized for detection in the SYBR® Green channel of qPCR instruments, and therefore low reaction volumes and low protein concentrations can be used.
- GloMelt™ dye is compatible with high concentrations of protein stabilizers (such as glycerol and sorbitol), and also protein destabilizers (such as DTT and imidazole).
- GloMelt™ dye performs very well in high detergent concentrations, unlike SYPRO® Orange.
- ROX dye can be included with GloMelt™ dye during thermal shift assays, which improves results by increasing replicate consistency in PCR instruments that require ROX passive reference dye.



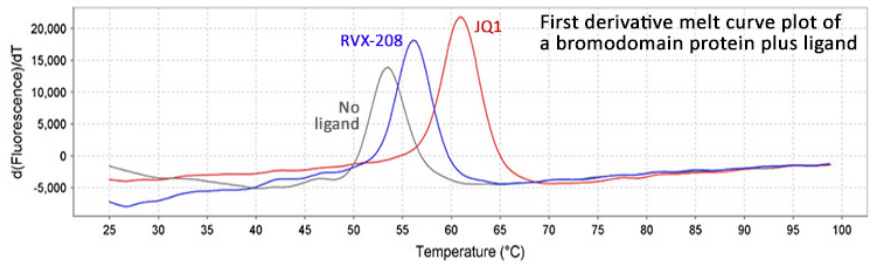
GloMelt™ performs well in the presence of detergent

Figure 2. IgG melt curve plots in the presence of detergent. A thermal shift assay was performed on 20 ug IgG in the presence of 5X SYPRO® Orange or 1X GloMelt™ dye, using a QuantStudio™ 5 qPCR system. The presence of detergent inhibited the SYPRO® Orange assay, but did not significantly affect the GloMelt™ curve.



Normalization with ROX reference dye can improve GloMelt™ results by increasing replicate consistency

Figure 3. A thermal shift assay was performed on 20 ug IgG in the presence 1X GloMelt™ dye and 50 nM ROX. After ROX normalization the standard deviation was reduced more than 5-fold.



Protein interactions with small molecule ligands can be detected

Figure 4. Small molecule inhibitors of bromodomain proteins have shown therapeutic effects in cancer models. Here, a GloMelt™ thermal shift assay was performed on 10 ug bromodomain BRD2 in the presence of bromodomain inhibitors (+)-JQ1 or RVX-208. Inhibitor binding stabilized the protein as indicated by the shift of the melting curves.

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