

Protein Thermal Shift Dye

Thermal and light stability

The Applied Biosystems™ Protein Thermal Shift™ product line, which includes the Protein Thermal Shift™ Dye and Software, offers a complete solution for performing differential scanning fluorimetry (DSF) on Applied Biosystems real-time PCR systems, for the analysis of protein thermal stability and ligand binding.

Because stability of the dye used for the Protein Thermal Shift application is essential, the thermal and light stability of the Protein Thermal Shift Dye was tested, independent of other factors in the assay, under three different conditions:

- **Low temperature (4°C), in the dark**—since most dyes are stored in laboratory refrigerators
- **Room temperature (~25°C), normal laboratory lighting**—since most robotic autoloader systems are not refrigerated
- **Room temperature (~25°C), in the dark**—since, in general, fluorescent dyes are photolabile

Methods

Methods for all three Protein Thermal Shift Dye stability experiments:

- Reactions were set up in clear Applied Biosystems MicroAmp™ 384-well plates (Cat. No. 4343814)
- Replicates contained 10 µg of lysozyme (Sigma, 10 mg/mL), a protein known for its robust stability, in buffer plus 1X dye from the Applied Biosystems Protein Thermal Shift Starter Kit (Cat. No. 4462263)
- Plates were sealed with Applied Biosystems MicroAmp Optical Adhesive Film (Cat. No. 4311971) and stored under different temperature and light conditions, depending on the experiment, until they were run on the Applied Biosystems ViiA™ 7 Real-Time PCR System
- One plate was run at each time point from time 0 to 8 hours or 24 hours

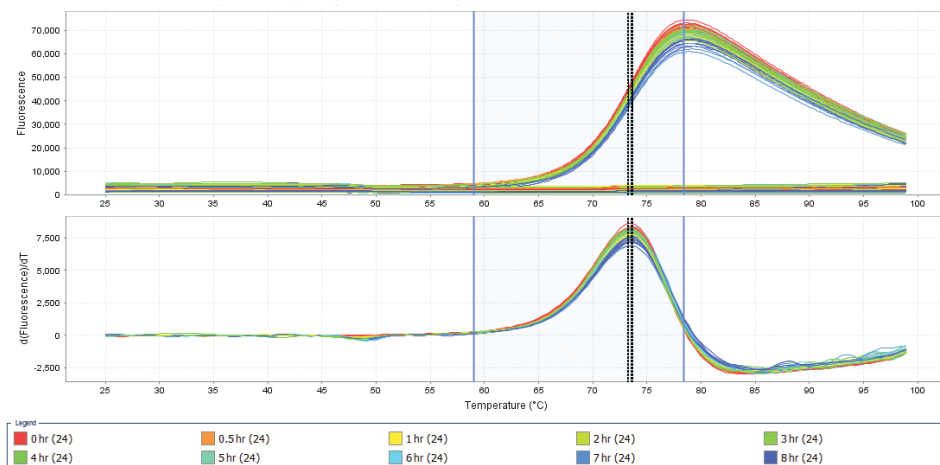


Figure 1. Dye stability results at low temperature in the dark. Ten assay plates with 24 replicate reactions were prepared and stored at 4°C in the dark for up to 8 hours prior to running them on the ViiA 7 Real-Time PCR System under conditions described in Methods. Here, a slight decrease in fluorescence intensity does not affect the T_m since it is calculated from the inflection point on the curve. T_m values through the 8 hr period range from average $T_m = 72.22 \pm 0.01^\circ\text{C}$ at time = 0, to average $T_m = 72.41 \pm 0.02^\circ\text{C}$ at time = 8 hr.

- Plates were run from 25°C to 99°C with a ramp rate of 0.05°C/sec

Dye stability results

Low temperature, in the dark

Results after storage of reactions at 4°C and in the dark show that the Protein Thermal Shift Dye is stable enough to not significantly affect T_m values for at least 8 hours, with a modest decrease of 10% in fluorescence intensity (Figure 1).

Room temperature, normal lighting

Results after storage of reactions at room temperature and with normal lighting indicate that the dye is stable for about 7 hours, but the level of fluorescence decreases dramatically over this period of time (Figure 2). These data also show that a decrease in fluorescence does not have a corresponding effect on T_m , since fluorescence intensity does not change the inflection point of the curves.

Room temperature, in the dark

Under room temperature and dark conditions, results were dramatically different, showing that the Protein Thermal Shift Dye is stable at room temperature in the dark for up to 24 hours with only a minor decrease in fluorescence over that period of time and a minimal change in T_m for lysozyme (Figure 3).

Conclusion

In this study, we investigated the thermal and light stability of the Protein Thermal Shift Dye when assembled in a reaction together with buffer and protein. We observed that, with lysozyme, a relatively thermostable protein, the reactions are stable for at least 8 hours at 4°C, and 24 hours at room temperature, when stored in the dark. The Protein Thermal Shift Dye will lose intensity when incubated in the presence of normal laboratory lighting, showing complete loss of performance after 7 hours under these conditions. From these observations, we conclude that assay plates with Protein Thermal Shift Dye can be set up in advance

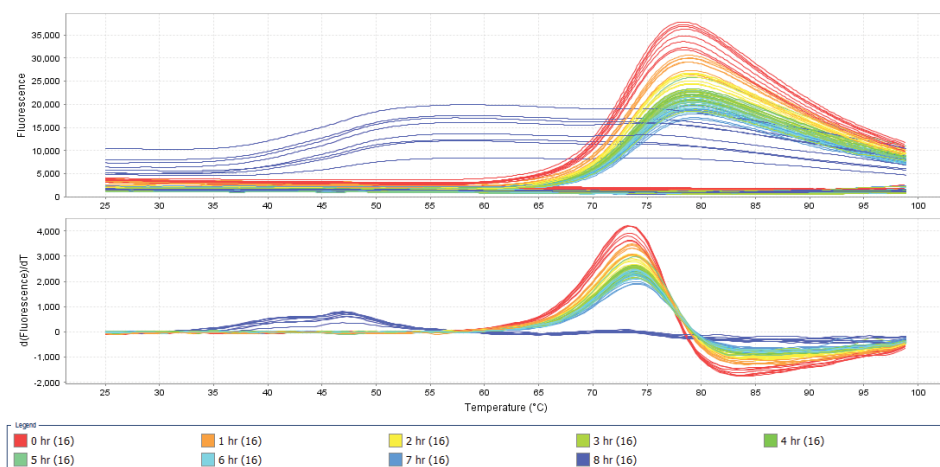


Figure 2. Dye stability results at room temperature in normal lighting Nine assay plates with 16 replicate reactions were prepared and stored at room temperature (~25°C) for up to 8 hours prior to running them on the ViiA 7 system under conditions described in Methods. The T_m values for this experiment were also consistent during the first 7 hours. T_m values slightly shifted from average $T_m = 72.09 \pm 0.02^\circ\text{C}$ at time = 0 hr, to average $T_m = 72.90 \pm 0.03^\circ\text{C}$ at time = 7 hr. At time = 7 hr, the Protein Thermal Shift Dye had only half as much fluorescence intensity as it did at time 0 hr, but this is enough to obtain T_m results. After 8 hours of storage at room temperature, under normal laboratory lighting, the Protein Thermal Shift Dye seemed to have degraded, and the normal lysozyme signal was no longer present, as shown by the dark blue lines.

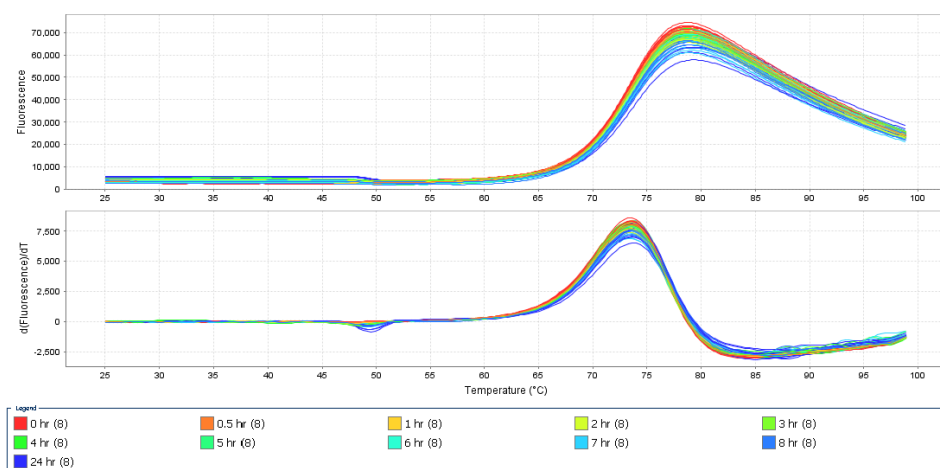


Figure 3. Dye stability results at room temperature in the dark Eleven assay plates with 8 replicate reactions were prepared and stored at room temperature in the dark prior to running them on the ViiA 7 system under conditions described in Methods. Results after storage for 0–24 hours show a change of less than 0.34°C in the T_m of lysozyme over 24 hours, as measured by the Boltzmann fit method, as well as standard errors of ≤ 0.04 for each set of 8 replicates per time point.

for high-throughput experiments if the protein and ligands under study are stable at either room temperature or 4°C, and the reaction plates should preferably be kept in the dark before being run on the instrument. The limiting factor for benchtop stability of assembled reactions is therefore more likely to be the protein itself. Given that a Protein Thermal Shift run is typically

5 to 30 minutes long, automation is a viable strategy. Moreover, the Protein Thermal Shift Software (Cat. No. 4466037) has the capability of creating multi-plate studies, allowing for the data from multiple plates to be analyzed together and further enabling high-throughput analysis workflows.

Find out more at thermofisher.com/proteinmelt

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