applied biosystems®
by *life* technologies™

High-throughput analysis of protein thermal stability

Protein Thermal Shift™ software and reagent kits

- Use Applied Biosystems® real-time PCR instrumentation to analyze protein thermal stability

- Cost-effective method to screen for ligand–protein binding, optimal buffer conditions, or stability changes

- Simple and rapid method, with results in as little as 0.5–2 hours

The Protein Thermal Shift™ (PTS) solution, which includes Protein Thermal Shift™ reagents and software, offers a new application for Applied Biosystems® real-time PCR systems— the analysis of protein thermal stability, including high-throughput screening of ligand–protein binding, optimization of buffer conditions that promote protein stability, and the effect of mutations or modifications on a protein's thermal stability.

Proteins are the key molecule studied as targets for new drugs as part of the drug development lead generation process. Drug discovery involves high-throughput screening of thousands of small molecules and ligands with a variety of different assays, requiring many months of time as part of a lead generation program. Protein targets are also a challenge to work with due to their susceptibility for degradation and aggregation—requiring the addition of

protein stability screening without ligand as well. Beyond drug discovery, protein thermal stability screening, which is performed with protein melting techniques, is also employed in many other research programs that utilize native proteins throughout academia and industry. For example, the identification and use of ligands and/or solution (buffer) conditions that maximally stabilize a protein are utilized as part of protein purification, crystallization, and functional characterization.

Protein thermal shift assay technology can improve the success rates of protein purification and crystallization and reduce the cost of drug discovery, offering a rapid, cost-effective, high-throughput screening method compared to other available technologies. The Protein Thermal Shift™ application, run on our real-time PCR systems, enables you to inexpensively and efficiently:

- Screen samples for relative protein thermal stability
- Screen samples for ligand binding to proteins in a high-throughput manner
- Screen for small-molecule and fragment-library drug candidates that bind to your protein target
- Screen for antibodies that bind to your protein target
- Screen samples for stability changes after protein point mutations
- Systematically identify suitable/optimal buffer conditions to measure protein–ligand interactions
- Identify optimal buffer conditions to improve protein purification and preparation
- Screen buffers to identify conditions for successful crystallization
- Screen buffers to identify optimal storage conditions for proteins

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How does Protein Thermal Shift™ technology work?

Protein stability is dependent on buffer pH, salt content, and the presence of various cofactors in a protein's storage or reaction buffer environment. A real-time melt experiment with a protein binding dye such as the Protein Thermal Shift™ Dye, run on any Applied Biosystems® real-time PCR system (including StepOne™, StepOnePlus™, 7500, 7500 Fast, and ViiA™ 7 systems) yields a fluorescence profile specific to the protein of interest in a given test buffer environment. Variations in the pH, salt content, or test buffer components are reflected in relative changes to this fluorescence profile and the T_m (melting temperature) calculated from it. The binding of a ligand to a protein also has a stabilizing effect on the protein's thermal stability, thus leading to a measurable difference in the protein's fluorescence profile.

Protein Thermal Shift™ Assay

The Protein Thermal Shift™ reagents enable a protein melt assay that is an

Steps in a Protein Thermal Shift™ Assay

- Mix protein, buffer, ligand, if applicable, and Protein Thermal Shift™ Dye
- Run a melt curve experiment on an Applied Biosystems® real-time PCR instrument
- The protein unfolds as it is heated
- The Protein Thermal Shift™ Dye binds exposed hydrophobic regions and fluoresces
- Transfer *.eds file to the Protein Thermal Shift™ software for analysis
- The melting temperature (T_m) is calculated from the melt curve
- Changes in T_m are correlated to changes in protein stability or ligand binding

efficient screening tool for measuring protein thermal stability, identifying suitable buffer conditions, and measuring protein–ligand interactions. This Protein Thermal Shift™ assay allows for systematic identification of optimal buffer conditions and ligands that stabilize proteins.

The Protein Thermal Shift™ assay is very easy to set up and run, and the entire workflow typically takes from 0.5–2 hours, depending on the system and run conditions used (Figure 1). The adjustable ramp rate and thermal accuracy of our real-time PCR systems allow complete flexibility to enable shorter run times and accommodate the requirements of screening workflows.

Protein Thermal Shift™ Software

Protein Thermal Shift™ Software has been developed to analyze protein melt fluorescence readings directly from Applied Biosystems® real-time PCR instrument files. Different proteins will have different thermal shift profiles, each with a unique melt curve shape, slope, signal-to-noise ratio, and temperature melt range. The Protein Thermal Shift™ Software generates one or multiple melting temperatures (T_m values) from these curves by the following two methods: Boltzmann-derived T_m and derivative curve–determined T_m. The Boltzmann T_m values are taken from the inflection
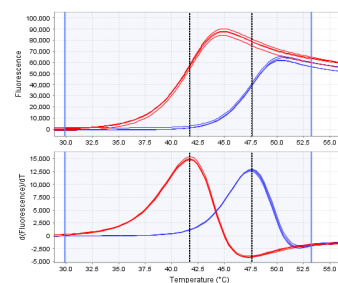

0.5—2 hours
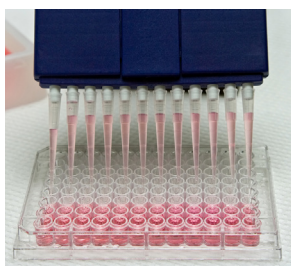
1	2	3
Prepare assays	Run melt assay on real-time PCR system (StepOne™/StepOnePlus™, 7500 and 7500 Fast, ViiA™ 7 system)	Analyze data with PTS software

Mix protein, buffer, ligand (if applicable), and PTS dye.

The protein unfolds with increasing temperature, and the PTS dye binds to expose hydrophobic regions and fluoresces.

The melting temperature (T_m) is calculated from the melt curve; changes in T_m are correlated to changes in protein stability or ligand binding.

Figure 1. Protein Thermal Shift™ workflow.

point of the fluorescence melt curve plot (Figure 2, top panel) and the derivative T_m values are taken from the top of the peak in the derivative plot (Figure 2, bottom panel).

The Boltzmann method fits the data within an automatically (or manually) identified melt region to the two-state Boltzmann model to generate the T_m. Typically, for proteins with a single melt domain, the Boltzmann method is used. However, for proteins with multiple melt domains, the derivative method can be utilized to determine up to six T_m values per sample. The derivative method uses a numerically computed second derivative of the raw data to estimate the temperatures where up to six peaks (local maxima) may occur in the derivative profile. An empirically derived threshold on the signal-to-noise ratio is used to determine which local maxima will be detected.

Case study: protein–ligand binding

Protein researchers use a variety of methods to study protein stability and screen for ligands, including biosensors and other high-throughput screening (HTS) methods, as well as lower-throughput calorimetry and circular dichroism systems. These methods can yield a great level of detail for the protein under study, but range from being very slow and requiring large quantities of sample protein (calorimetry and circular dichroism) to fast but expensive (biosensors). Protein Thermal Shift™ technology fills the need for fast and inexpensive screening of samples in a high-throughput fashion to quickly narrow down the number of candidates that merit more detailed studies with other technologies. The flexibility, speed, and ease-of-use of the Protein Thermal Shift™ solution makes it an excellent option for protein researchers who need to

understand how the stability of their proteins is affected at all stages of their research.

Figure 2 demonstrates the identification of a protein-binding ligand, as shown by the increase in T_m of the protein when bound to a ligand. The red curves represent replicate measurements of the protein in solution, while the blue curves represent replicate measurements of the same protein after incubation with a ligand that binds to the protein. For this sample the T_m shifted from ~41.5ºC to 47.5ºC, indicating that the protein stability increased upon ligand binding.

Summary

Protein researchers use a number of methods to study protein stability and screen for ligands. These methods are generally very slow and tedious to perform and require large quantities of sample protein. Some fast methods exist, but they are very expensive to run compared to the Protein Thermal Shift™ Assay.

Protein Thermal Shift™ reagent kits utilized on Applied Biosystems® real-time PCR systems enable fast and inexpensive screening of samples in a high-throughput fashion to quickly narrow down the number of candidate ligands or buffer conditions for a wide range of applications that are impacted by protein stability.

Protein Thermal Shift™ software allows researchers to quickly compare the shift in T_m (delta T_m or ΔT_m) between different assay conditions or different ligands added to a sample, relative to a reference sample, thus providing a tool to screen and identify conditions that stabilize (or destabilize) a protein or to screen ligands that bind to the protein of interest.
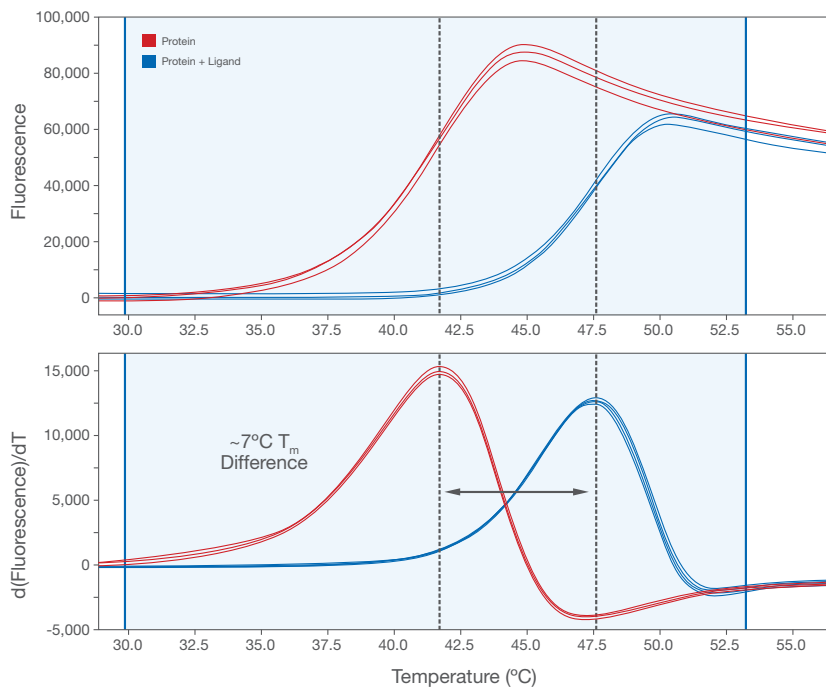

Figure 2. Protein stabilization upon ligand binding. The protein T_m increases after a ligand is added. (**Top panel**) Fluorescence melt curve plot. (**Bottom panel**) Derivative curve plot.

Compatible instruments

System	System features	Part No.
StepOne™ Real-Time PCR System	• Capacity: 48-well plates, 0.1 mL tubes • Fast block: yes • Colors: 3	4376357
StepOnePlus™ Real-Time PCR System	• Capacity: 96-well plates, 0.1 mL tubes • Fast block: yes • Colors: 4	4376600
7500 Real-Time PCR System	• Capacity: 96-well optical plates or 0.2 mL tubes • Fast block: no • Colors: 5	4351104
7500 Fast Real-Time PCR System	• Capacity: 96-well optical Fast plates • Fast block: yes • Colors: 5	4351106
ViiA™ 7 Real-Time PCR System	• Capacity: 96-well, 384-well, TaqMan® Array Micro Fluidic Cards • Fast block: yes • Colors: 21	4453534

Ordering information

Reagent kits	Description	Part No.
Protein Thermal Shift™ Dye Kit	This kit includes the following components: Protein Thermal Shift™ Dye (2,000 reactions) Protein Thermal Shift™ Buffer (2,000 reactions)	4461146
Protein Thermal Shift™ Starter Kit	This kit includes the following components: Protein Thermal Shift™ Dye (2,000 reactions) Protein Thermal Shift™ Buffer (2,000 reactions) Protein Thermal Shift™ Control Ligand (100 reactions) Protein Thermal Shift™ Control Protein (100 reactions)	4462263

Protein Thermal Shift™ Software	Description	Part No.
Protein Thermal Shift™ Software version 1.0	1 kit—10 licenses	4466037
Protein Thermal Shift™ Software additional license	1 license—separate software purchase required	4466038

Protein Thermal Shift™ Studies Application User Guides	Description	Part No.
User Documentation Set	1 User Guide and 3 Quick Reference (4 manuals, printed)	4461808
Ligand Screening Quick Reference	1 manual	4461812
Buffer Screening Quick Reference	1 manual	4461810
Mutation Screening Quick Reference	1 manual	4461811

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