applied biosystems
by life technologies

Protein Thermal Shift™ Dye Kit

Pub. Part no. 4461806 Rev. B **Rev. Date** May 2011

Catalog no.	Quantity	Component	Storage conditions
4461146	Sufficient for 2000 reactions	Protein Thermal Shift™ Buffer	Room temperature (RT, 18°C to 25°C)
		Protein Thermal Shift™ Dye	

Note: For safety and biohazard guidelines, refer to the "Safety" section in the *Protein Thermal Shift™ Studies User Guide* (PN 4461808). For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Refer to the *Protein Thermal Shift™ Studies User Guide* (PN 4461808) for detailed instructions on preparing and running the protein melt reactions, using the Protein Thermal Shift™ Starter Kit, reviewing the results, and troubleshooting.

Prepare the protein melt reactions

We recommend that you prepare four replicates of each reaction.

1. Prepare a fresh dilution of Protein Thermal Shift™ Dye (1000×) to 8×.
2. Place the reaction plate on ice, then add reaction components to the plate in the order listed.

Component	Volume
Protein Thermal Shift™ Buffer	5.0 µL
Water + protein (0.1 to 1 mg/mL stock) + buffer or ligand	12.5 µL
Diluted Protein Thermal Shift™ Dye (8×)	2.5 µL
Total volume for each reaction	**20.0 µL**

3. Pipet up and down 10 times to mix well.
4. Seal the plate with MicroAmp® Optical Adhesive Film, spin it at 1000 rpm for 1 minute, then place it on ice.

Set up and run the real-time PCR instrument

IMPORTANT! Keep the protein melt reactions on ice until you load the instrument.

1. Using the real-time PCR instrument software, open and set up the experiment run file:

Setup	Setting
Experiment properties	• Experiment type: **Melt Curve** • Reagents: **Other** • Ramp speed: **Fast** or **Standard**
Target properties	• Reporter: **ROX** • Quencher: **None**
Plate layout	• Assign targets to all wells in use • Passive reference: **None**
Run method	• Reaction volume per well: **20 µL** • Ramp mode: **Continuous** • Thermal profile: Step 1, Temp: **25**°C, Time: **2 minutes** Step 2, Temp: **99**°C, Time: **2 minutes** • Ramp rate: – ViiA™ 7 Real-Time PCR System: Step 1: **1.6**°C**/s**, Step 2: **0.05**°C**/s** – StepOne™, StepOnePlus™, 7500, and 7500 Fast Systems: Step 1: **100%**, Step 2: **1%** • (ViiA™ 7 System only) Optical filters: – Excitation filter: **x4(580±10)** – Emission filter: **m4(623±14)**

2. Load the plate, then start the instrument run.

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

For support visit www.appliedbiosystems.com/support

www.lifetechnologies.com

life technologies™