

# 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 (1000X) to 8X.
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 (8X)	2.5 µL
<b>Total volume for each reaction</b>	<b>20.0 µL</b>

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

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



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