

Protein Thermal Shift™ Solution

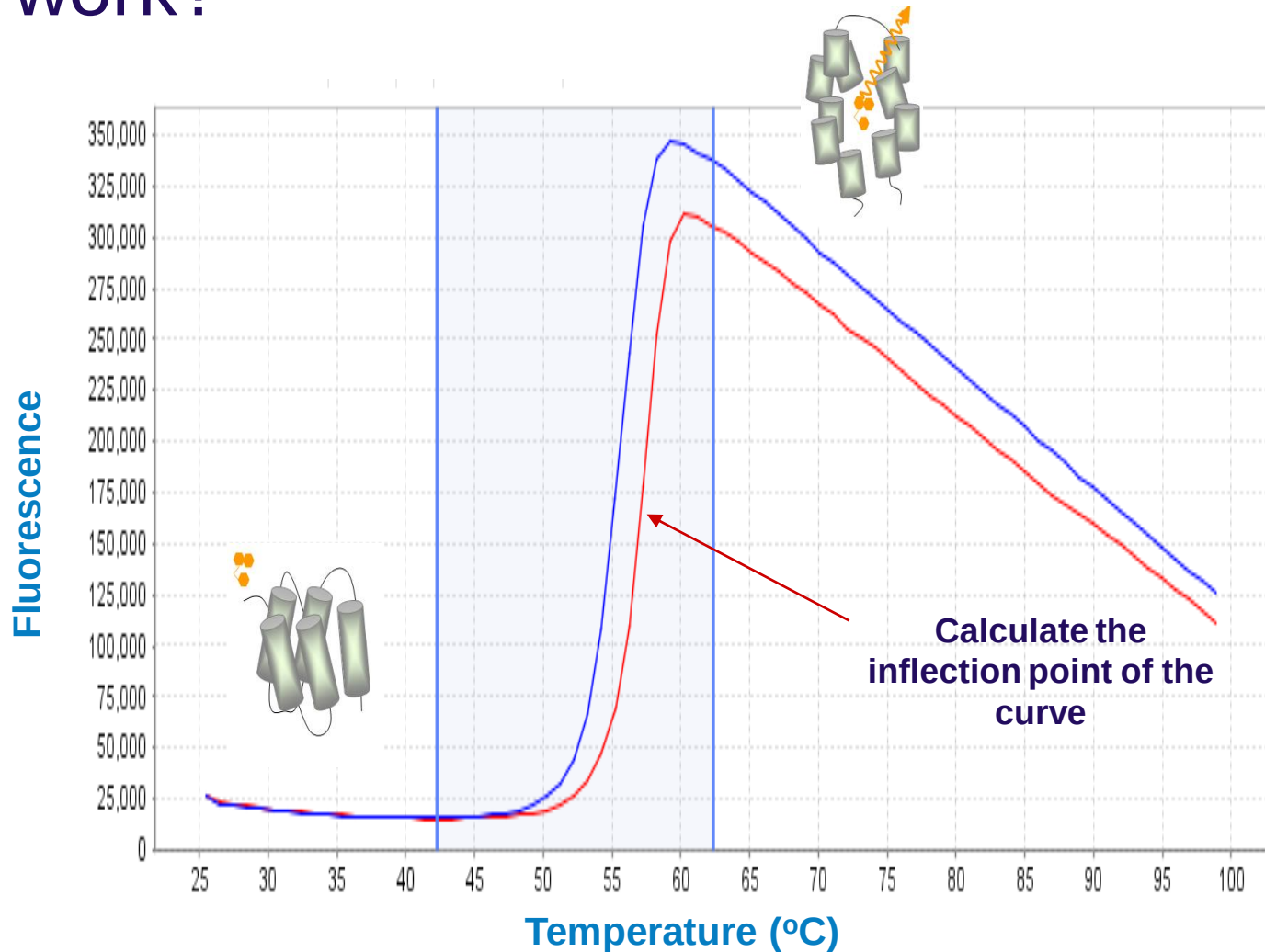
Using Applied Biosystems Real-Time PCR Systems

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Real Time PCR Systems



How does the Protein Thermal Shift™ technology work?

- The protein unfolds as it is heated.
- An environmentally-sensitive dye binds exposed hydrophobic regions and fluoresces.
- The T_m (melting temperature) is calculated from the melt curve.
- Changes in T_m are correlated to changes in protein stability.

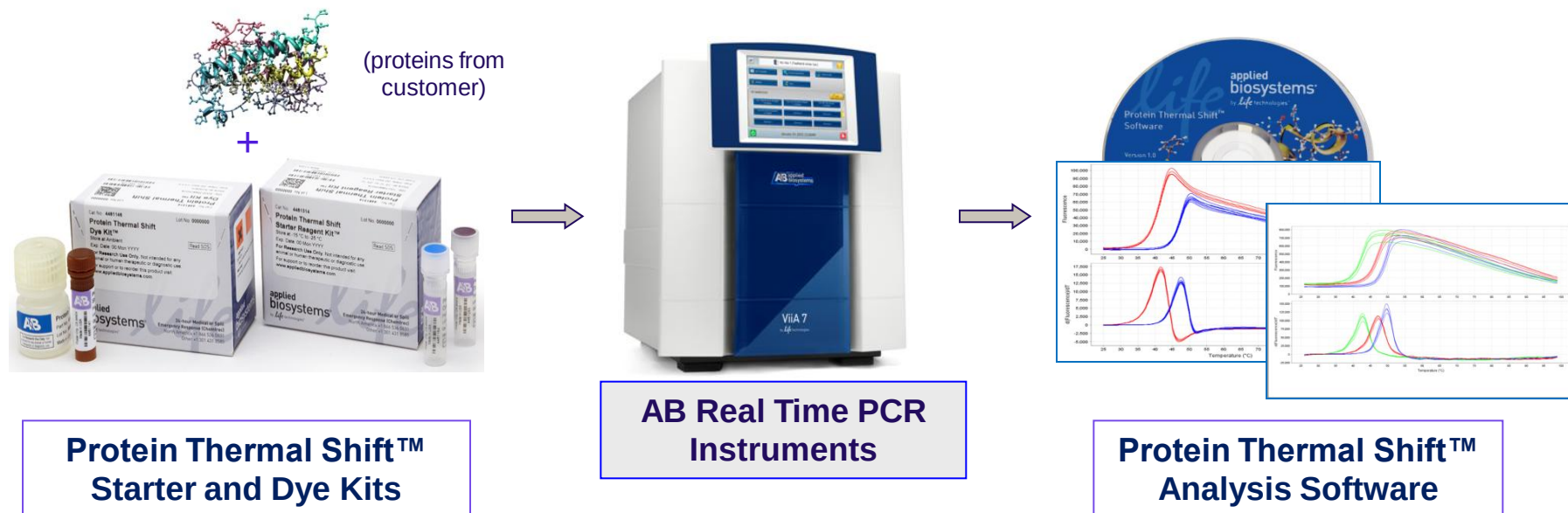


Protein Thermal Shift™ applications

- Protein Stability Screen:
 - improving protein preps (pH, salt, excipients)
 - profiling crystallization conditions
 - protein formulation and storage buffers
 - effect of mutations or modifications
 - Protein prep QC
- High-Throughput Ligand Screening:
 - Small-molecule and fragment screens
 - Antibody-target specificity
 - Protein-protein interaction
 - Inhibitor binding

Protein Thermal Shift™ Solution

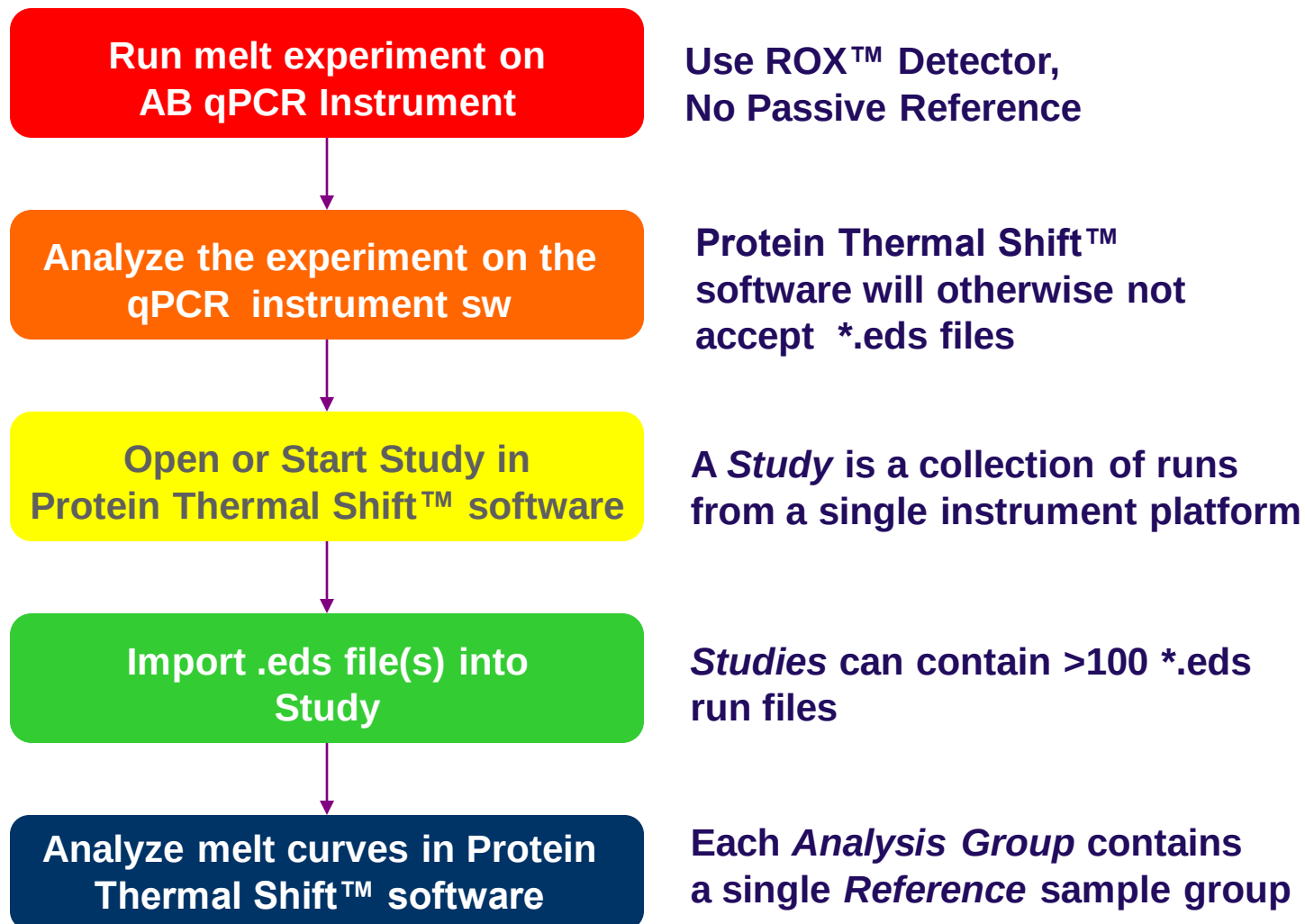
Reagents & Software enabling a new application for AB® qPCR instruments



Advantages of the Protein Thermal Shift™ Solution :

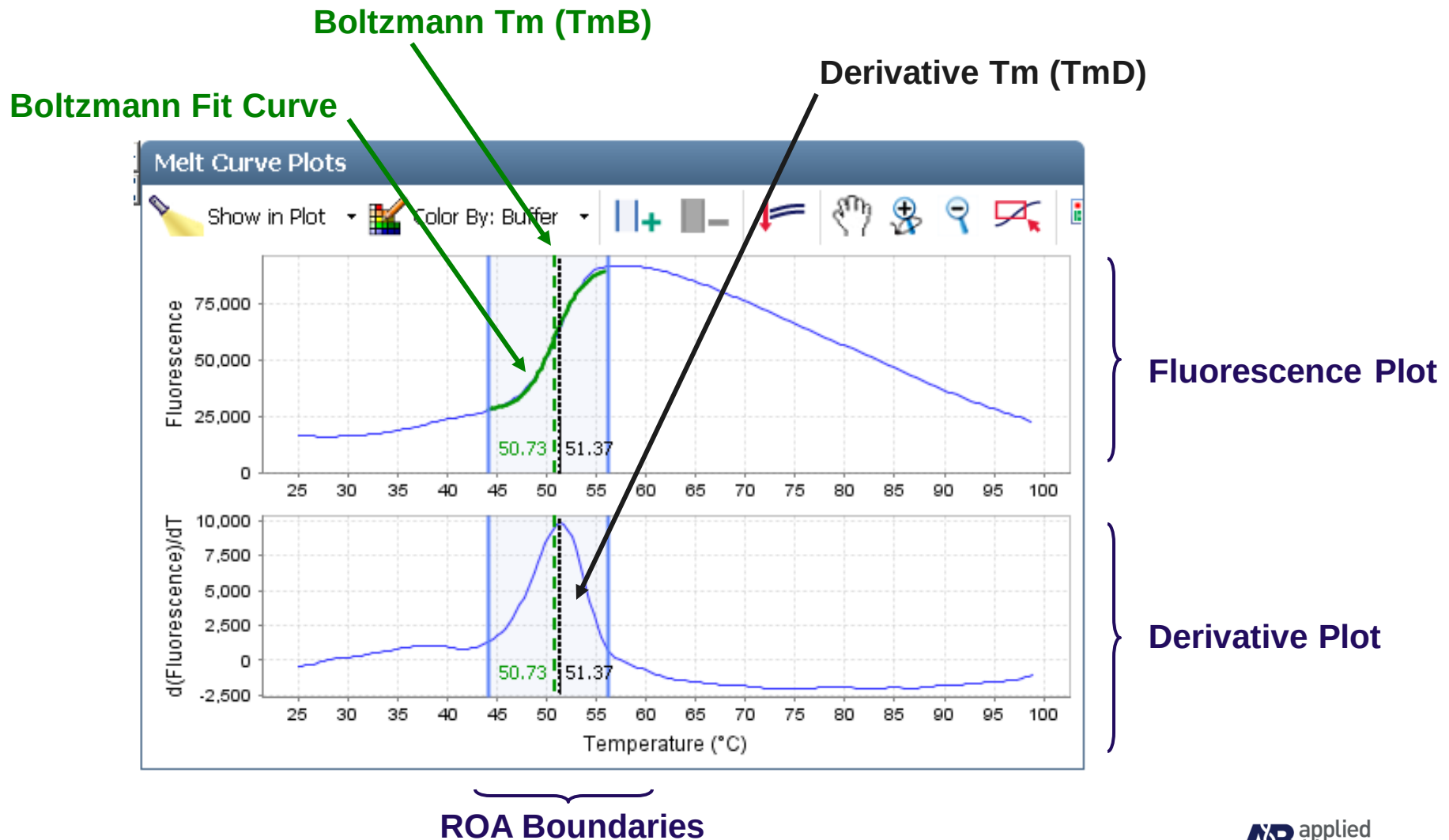
- High Throughput: 384 assays in < 30 minutes, robotics available
- Low sample requirements (usually <1 ug protein / assay)
- No *a priori* protein or ligand structure information necessary
- Minimal upfront optimization

Protein Thermal Shift™ basic workflow - Calibration



Protein Thermal Shift™ Software v1.0

Fluorescence and Derivative plots

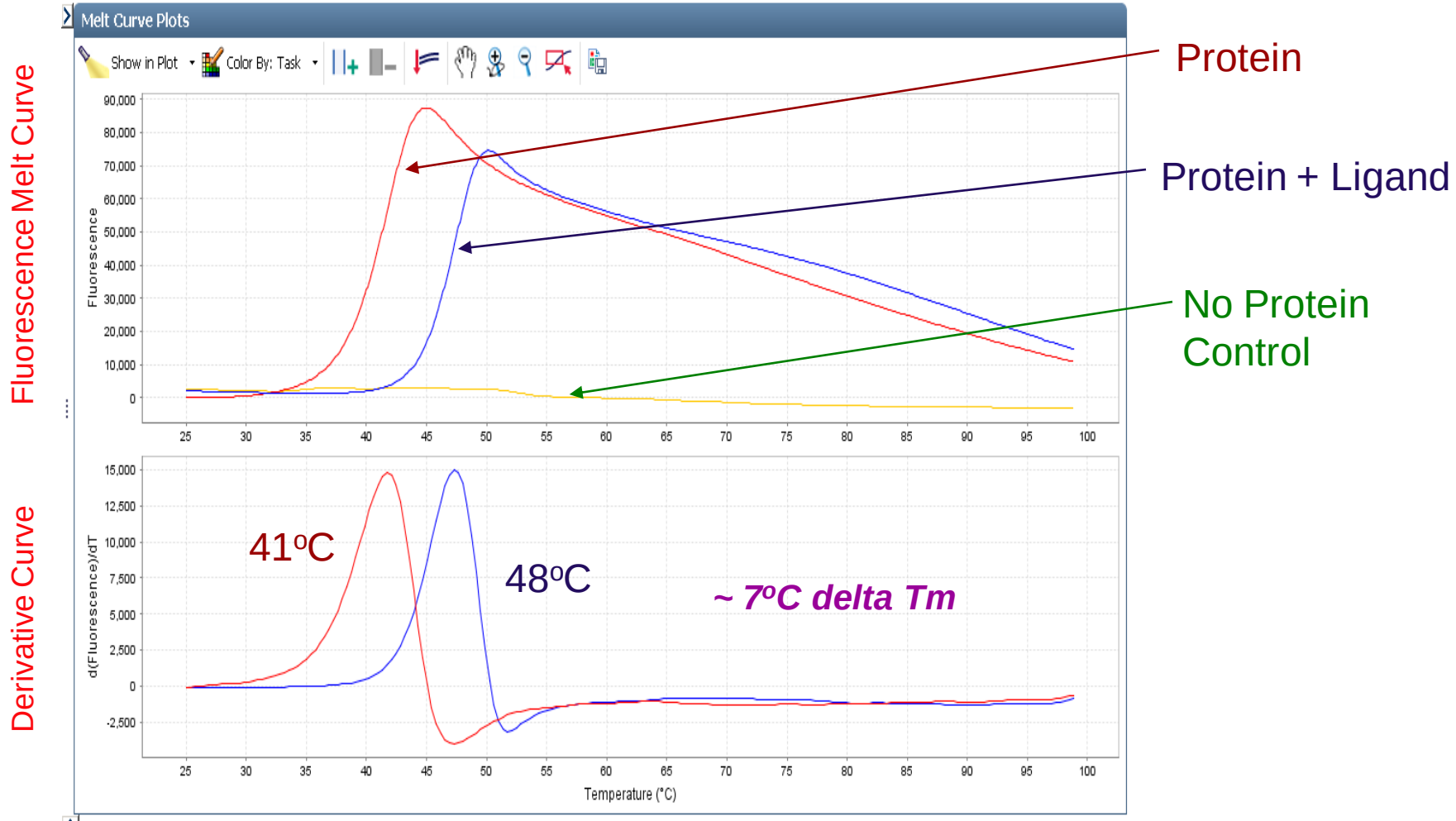


Drug Screening/Ligand Binding Application

- To assess shift of the unfolding temperature of the protein tested in presence of ligand relative to that obtained in the absence of ligand.
- Ligand used: ATP (0.0, 0.1, 1mM)
- Protein used: T4 DNA Ligase (0.1 mg/ml)
- DNA Ligase involved in DNA repair and replication
- ATP is required for the action of DNA Ligase
- 100mM Potassium Phosphate, pH 6.0, 150 mM NaCl

Protein-Ligand Binding:

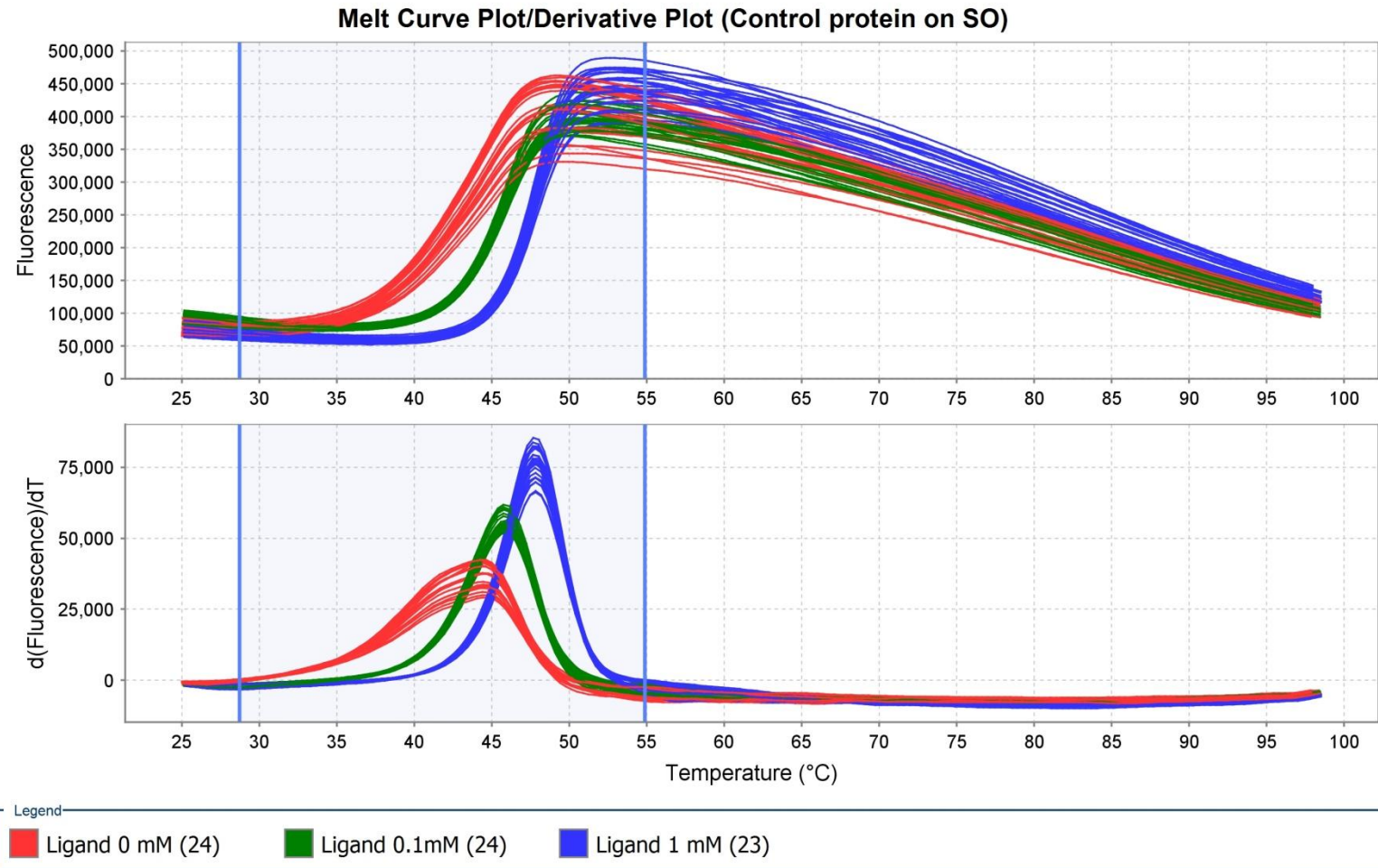
Increase in Protein Thermal Stability with bound Ligand



ViiTM7 Real Time PCR System

Protein-Ligand Binding:

Increase in Protein Thermal Stability with bound Ligand



Buffer Screening Application

- Material Tested, RecA (2mg/ml) -> New England BioLabs (M0249L)
- *E. coli* RecA is necessary for genetic recombination, reactions involving DNA repair and UV-induced mutagenesis
- Screening for appropriate buffer conditions to convey stability for protein storage and/or additional test conditions
- Four buffers are tested for the assay
- Each Buffer titrated with different concentration of NaCl
- Sixteen different buffers in total

Buffer Screening Application

	Buffer Screening plate set up								NTC			
	1	2	3	4	5	6	7	8	9	10	11	12
A	A1 Buffer	A2 Buffer	A3 Buffer	A4 Buffer	A1 Buffer	A2 Buffer	A3 Buffer	A4 Buffer	A1NTC	A2NTC	A3NTC	A4NTC
B	A1 Buffer	A2 Buffer	A3 Buffer	A4 Buffer	A1 Buffer	A2 Buffer	A3 Buffer	A4 Buffer	A1NTC	A2NTC	A3NTC	A4NTC
C	B1 Buffer	B2 Buffer	B3 Buffer	B4 Buffer	B1 Buffer	B2 Buffer	B3 Buffer	B4 Buffer	B1NTC	B2NTC	B3NTC	B4NTC
D	B1 Buffer	B2 Buffer	B3 Buffer	B4 Buffer	B1 Buffer	B2 Buffer	B3 Buffer	B4 Buffer	B1NTC	B2NTC	B3NTC	B4NTC
E	C1Buffer	C2 Buffer	C3 Buffer	C4 Buffer	C1Buffer	C2 Buffer	C3 Buffer	C4 Buffer	C1NTC	C2NTC	C3NTC	C4NTC
F	C1Buffer	C2 Buffer	C3 Buffer	C4 Buffer	C1Buffer	C2 Buffer	C3 Buffer	C4 Buffer	C1NTC	C2NTC	C3NTC	C4NTC
G	D1Buffer	D2 Buffer	D3 Buffer	D4 Buffer	D1Buffer	D2 Buffer	D3 Buffer	D4 Buffer	D1NTC	D2NTC	D3NTC	D4NTC
H	D1Buffer	D2 Buffer	D3 Buffer	D4 Buffer	D1Buffer	D2 Buffer	D3 Buffer	D4 Buffer	D1NTC	D2NTC	D3NTC	D4NTC

A. Sodium citrate pH 5.5

B. Potassium phosphate, pH 6.0

C. Potassium phosphate, pH 7.0

D. Hepes. pH 7.5

1. 0mM NaCl

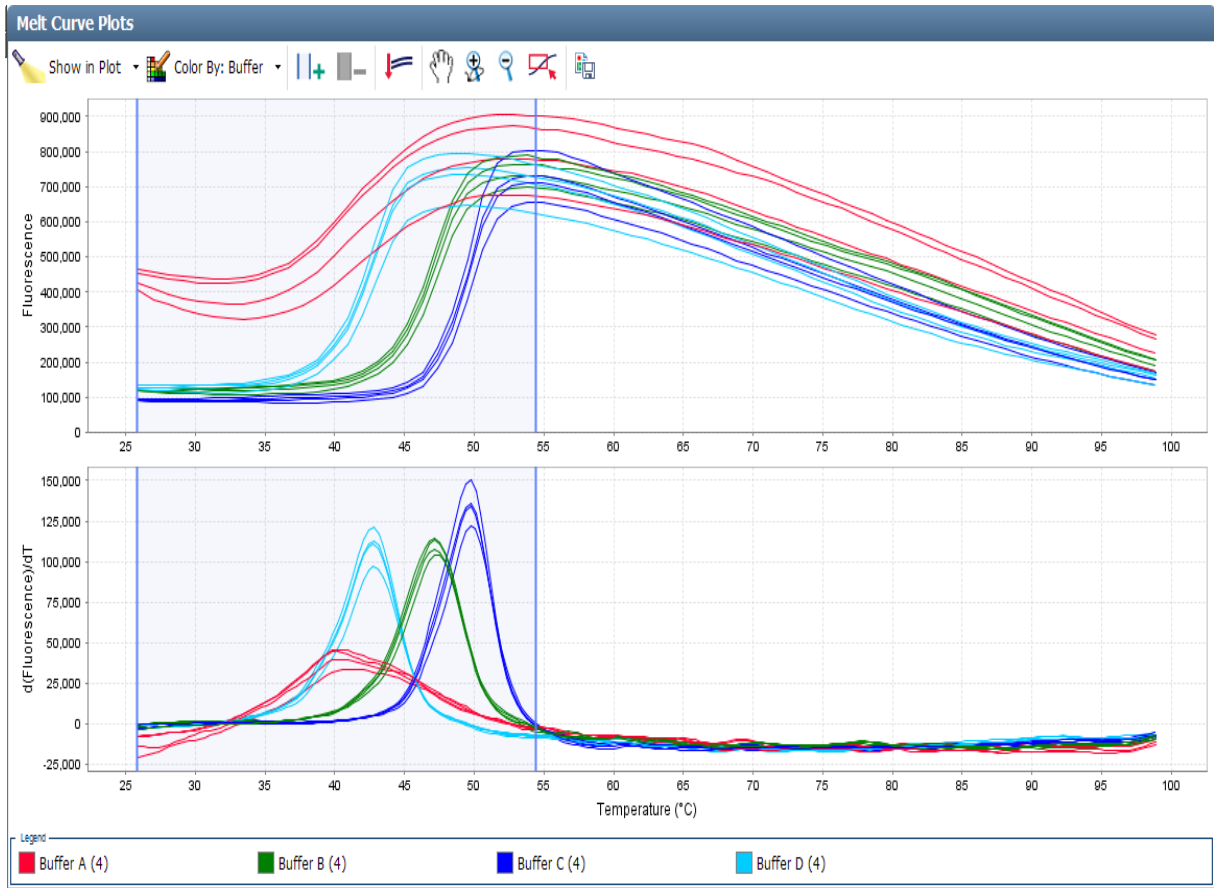
2. 50mM NaCl

3. 100mM NaCl

4. 150mM NaCl

Effect of Buffer Conditions on Protein Thermal Stability

Application: Buffer Screening with Rec A

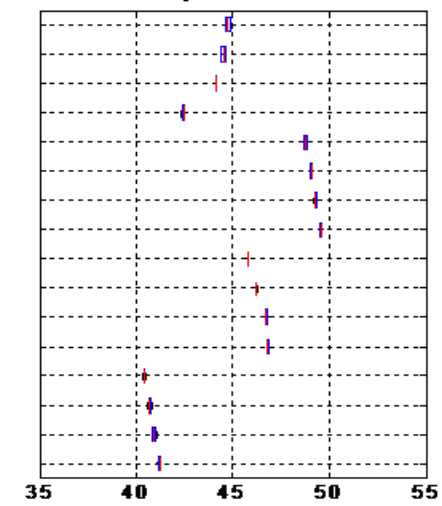


RecA BufferA4	38.6	-Na citrate pH 5.5 + 150 mM NaCl
RecA BufferB4	45.6	-KPO ₄ pH 6.0 + 150mM NaCl
RecA BufferC4	49	-KPO ₄ pH 7.0 + 150mM NaCl
RecA BufferD4	45	-Hepes. pH 7.5 + 150mM NaCl

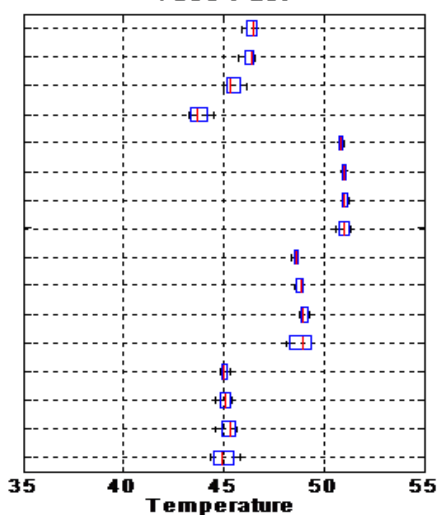
Buffer Condition

Buffer Condition

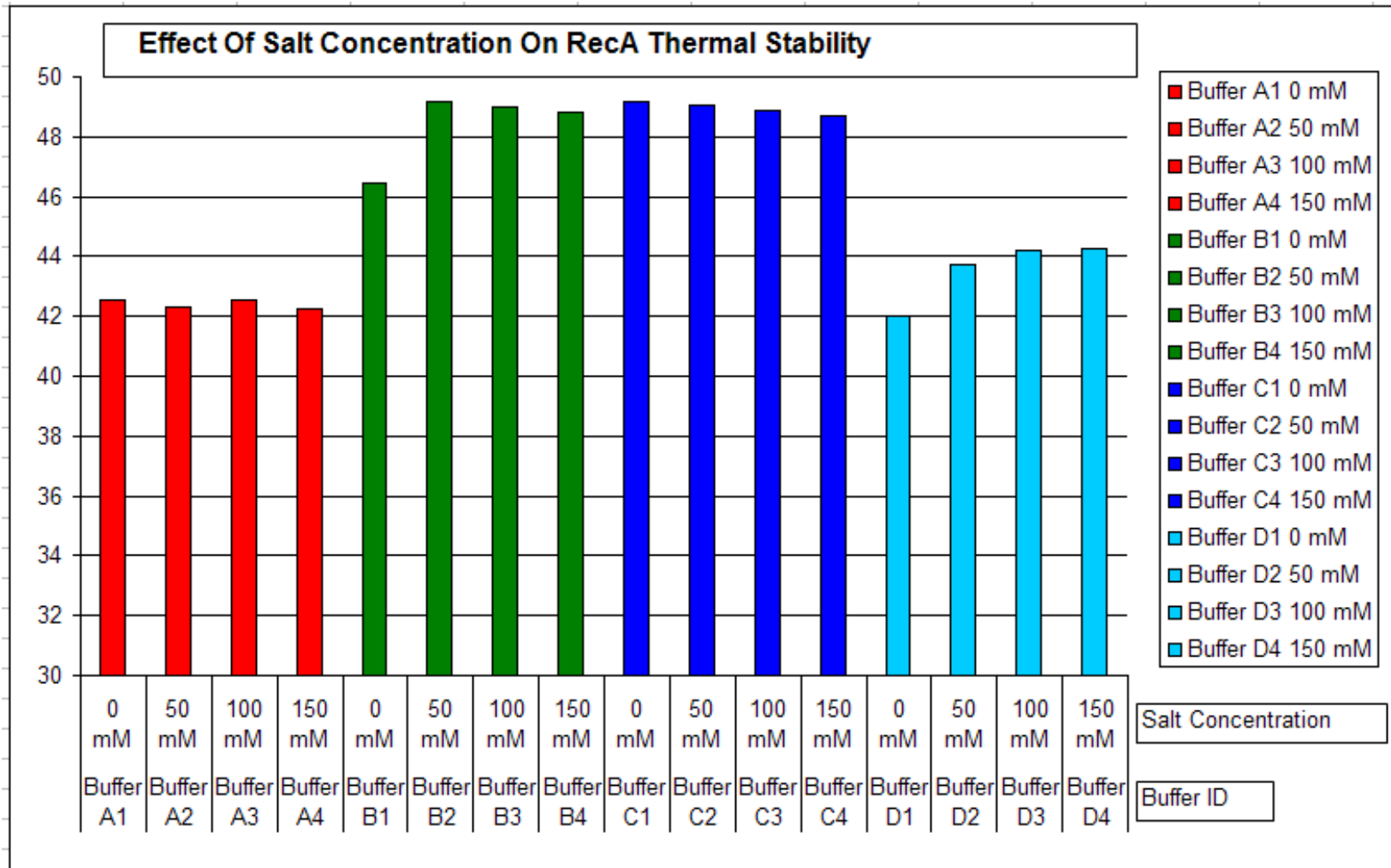
Step One Plus



7500 Fast



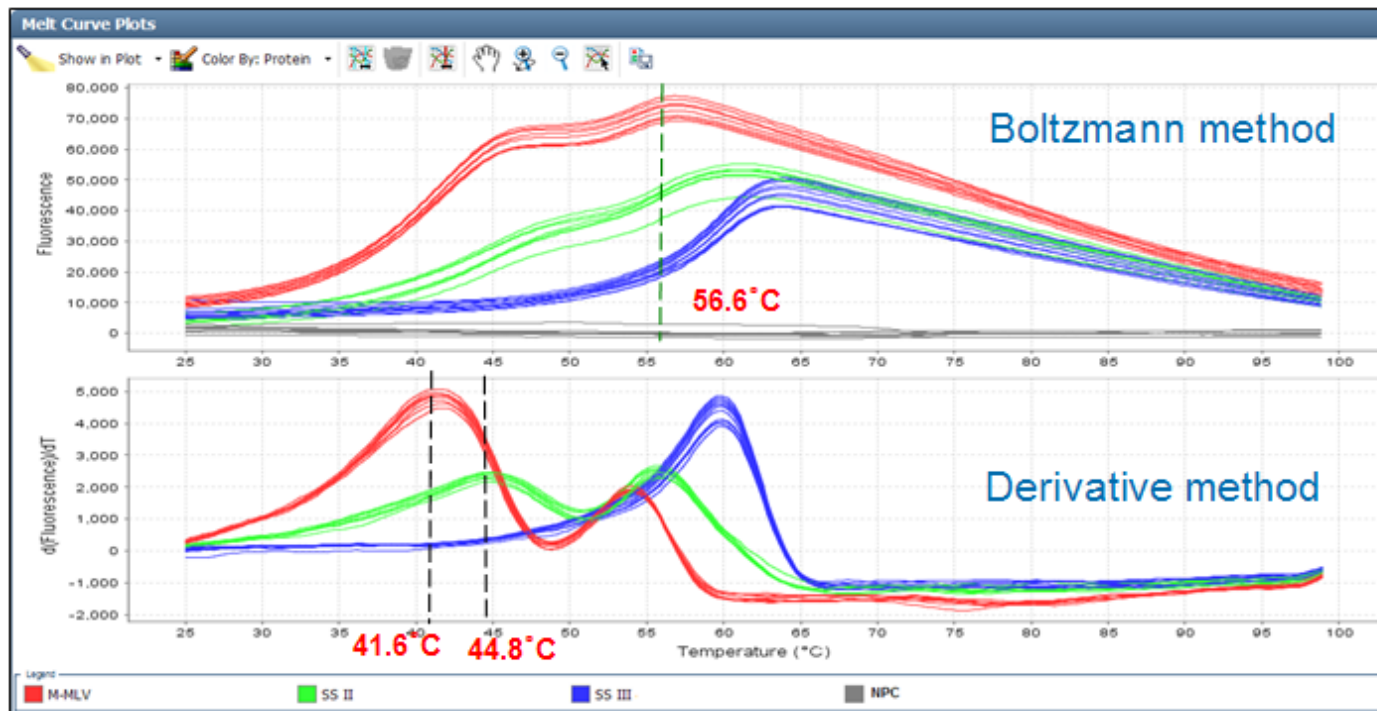
Effect of Buffer Conditions on Protein Thermal Stability



Mutation Screening Application

- SuperScript® II Reverse Transcriptase (RT) is an improved version of SuperScript® RT (M-MLV)
- SuperScript® II contains point mutations in the RNase H active center for reduced RNase H activity
- SuperScript® II mutations within the RNase H domain maintain full polymerase activity
- Full enzyme activity remains at 42°C
- SuperScript® III has further mutations for added thermostability

Effect of Point Mutations on Protein Thermal Stability

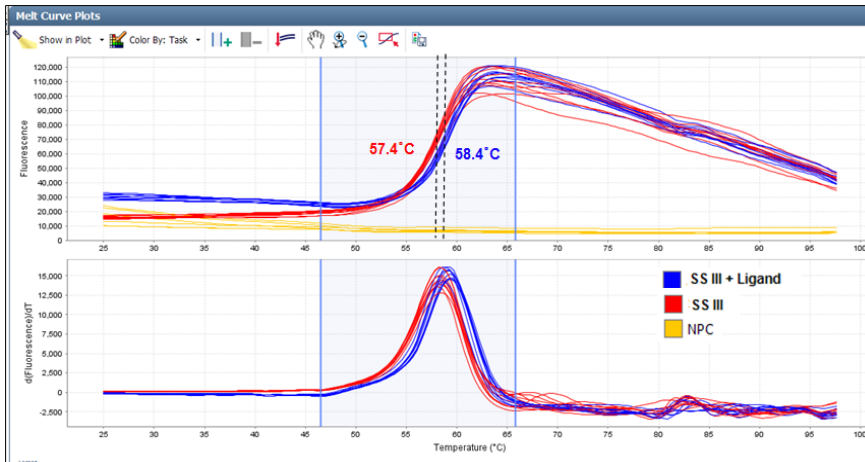
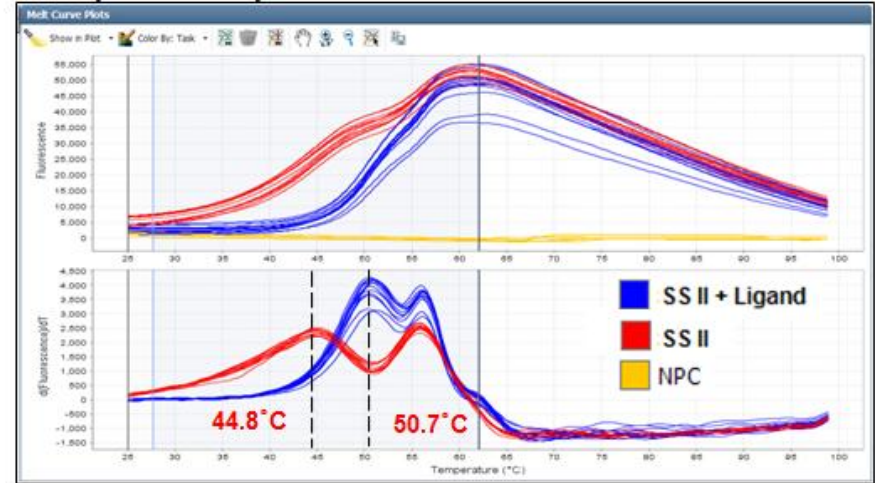
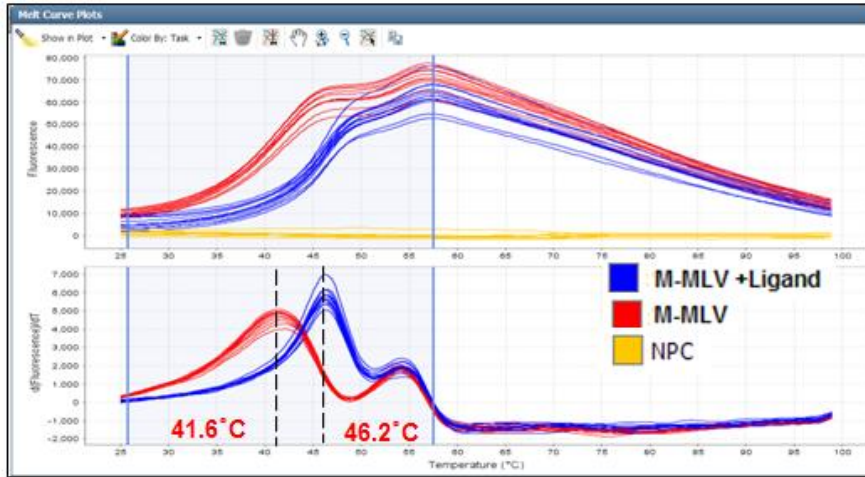


PTS Results

M-MLV	DTm: 41.6°C
SS II	DTm: 44.8°C
SS III	BTm: 56.6°C
NPC	

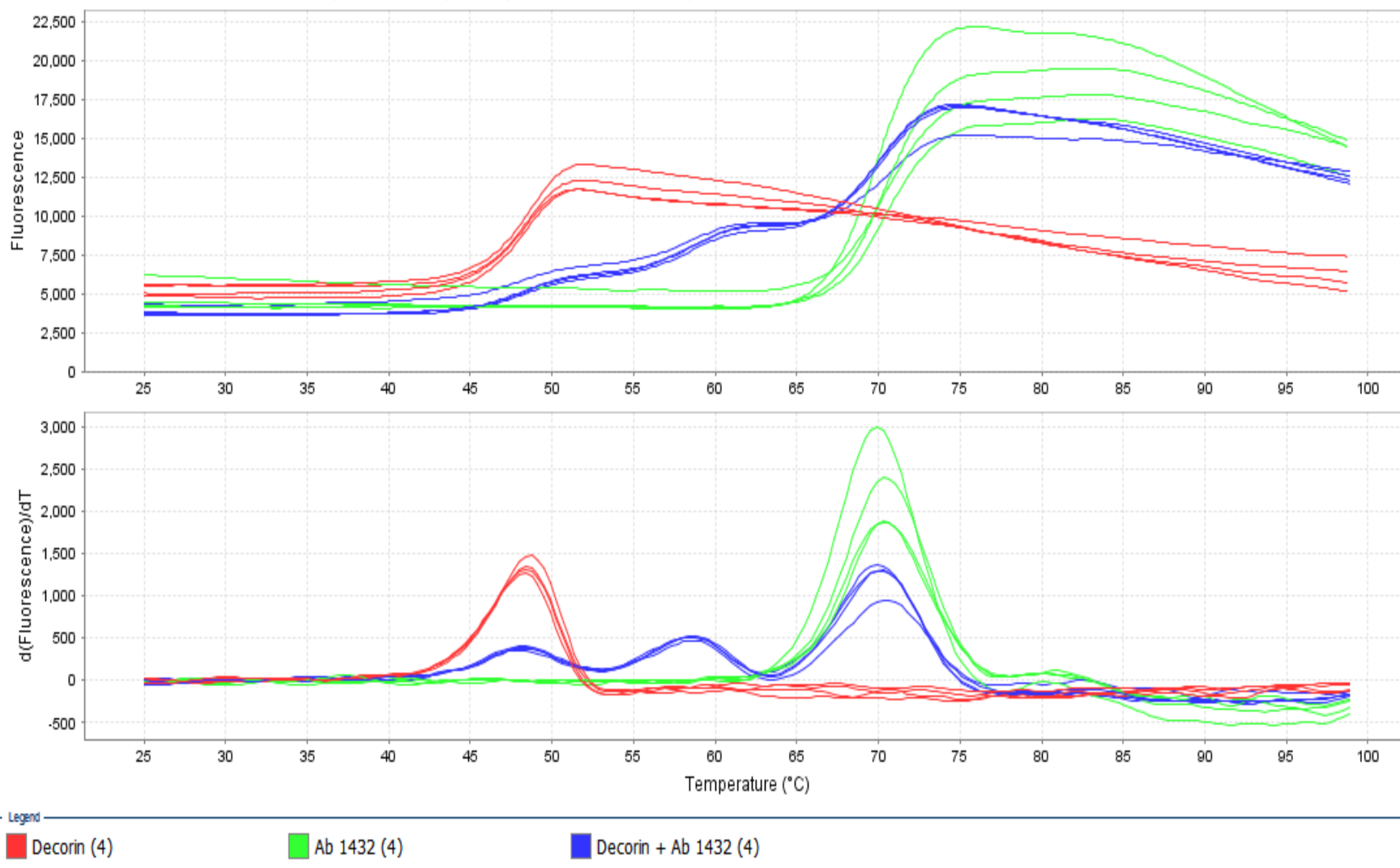
Protein Thermal Shift™ data from ViiA™ 7 instrument showing the Fluorescence and Derivative Melt profiles

Effect of Point Mutations on Protein Thermal Stability



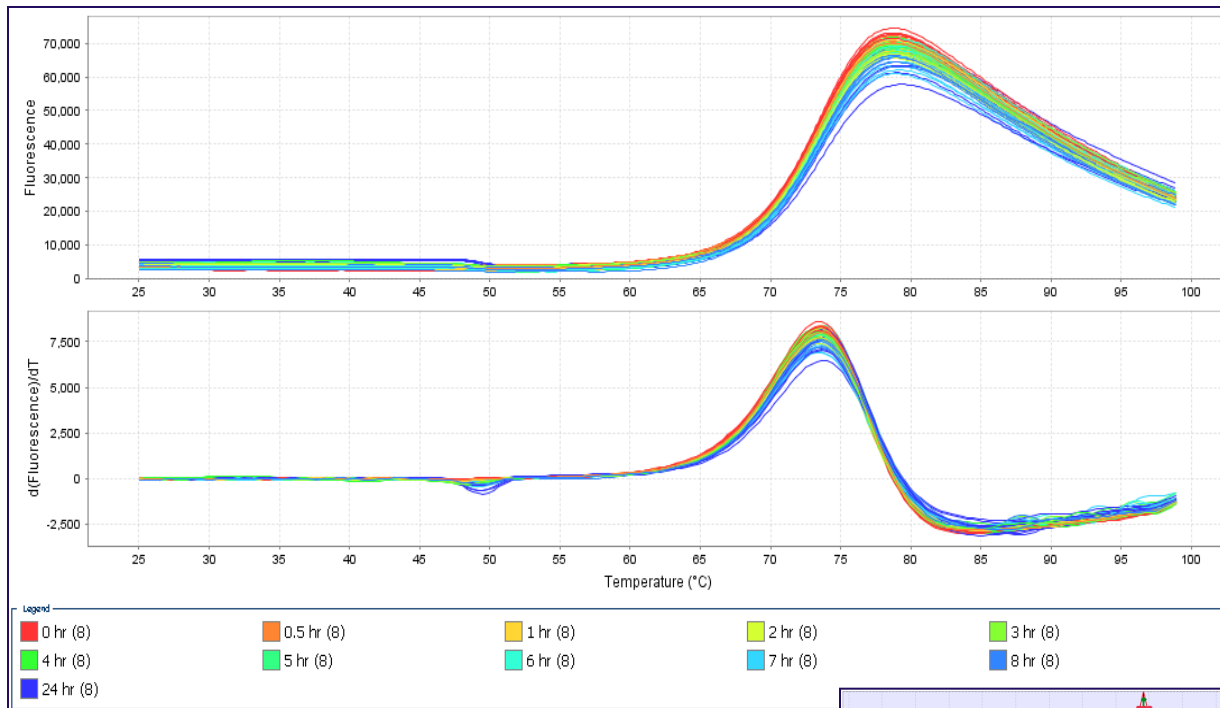
Protein Thermal Shift™ data from ViiA™7 instrument showing the Fluorescence and Derivative Melt profiles for M-MLV, SSII, SSIII (RED curves) plus ligand (BLUE curves, DNA oligonucleotide)

Antibody Binding increases Protein Thermal Stability

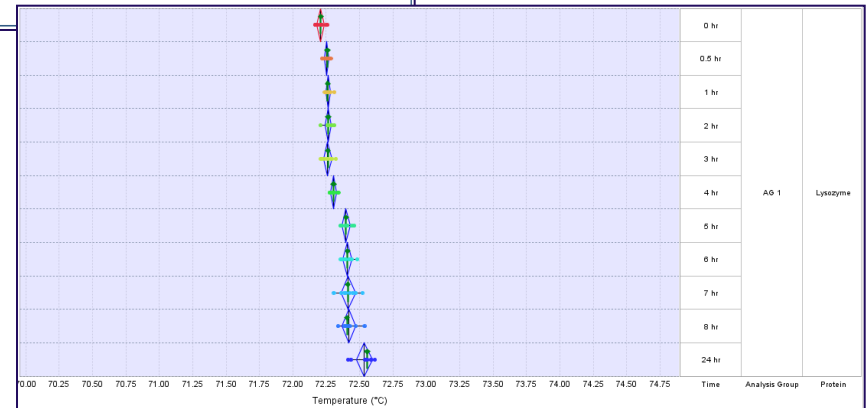


ViiA™7 Real Time PCR Instrument

Protein Thermal Shift™ Dye Stability



Lysozyme incubated at Room Temp in the dark for up to 24hrs with PTS dye.
 Run under standard conditions on the ViiA™7 at 0.05°C/sec. $\Delta T_m \leq 0.34^\circ \text{C}$ over 24 hours, standard errors of ≤ 0.04 for each set of 24 replicates per time point.
 Run on a ViiA™7 Real-Time PCR Instrument.



Conclusions

- The Protein Thermal Shift™ Assay is a rapid, inexpensive, and straight-forward high-throughput tool for screening conditions that maximize protein stability or libraries of ligands.
- Advantages:
 - Adjustable ramp speed and accurate temperature range flexibility.
 - Small reaction volumes, providing fast and accurate results with < 1µg of protein.
- Protein TSA has been performed on the Applied Biosystems 7500 Fast, StepOnePlus™, and ViiA™ 7 Real Time PCR Instruments, expanding the flexibility of these systems to protein research.
- Applied Biosystems' complete Protein Thermal Shift™ solution covers the whole range from dye reagent to instrumentation and analysis software.
- Trial software download at www.appliedbiosystems.com/proteinmelt

Acknowledgements

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