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Application Note

Application topics:

- Nucleic acid extraction and enrichment
- Standard, rapid and real-time PCR
- Kits and reagents
- Automated liquid handling
- Molecular, cellular and in-vitro assays
- Sample preparation and BioBanking
- Protein research
- Homogenization
- Spectrophotometry
- BioImaging
- Electrophoresis and blotting
- Others

Thermal shift assays using Jena Bioscience JBScreen Thermofluor Fundament and Specific Screens on Analytik Jena's qTOWER³

In protein research, investigating protein thermostability has become an essential approach as it is directly correlated to its molecular structural integrity and of crucial importance to protein downstream processes. For this, thermal shift assays have to be carried out against many different buffer conditions, which can turn into a very time consuming task with low reproducibility when conditions have to be manually prepared. Jena Bioscience has therefore developed two ready-to-use 96-well based screens that systematically evaluate the effect of ionic strength, pH and ions on protein's thermostability. The JBScreen Thermofluor FUNDAMENT analyses the effect of the fundamental factors pH and ionic strength on thermostabiliy while the JBScreen Thermofluor SPECIFIC screens for several metal ions of different valencies under different pH-conditions. Both screens are based on "Super Buffers", allowing a straightforward non biased identification of stabilizing factors.
Here we present JBScreen Thermofluor FUNDAMENT and SPECIFIC performed in Analytik Jena's real-time PCR cycler qTOWER³ at assay volumes down to 5µl.

Materials and Methods

Chemicals

- JBScreen Thermofluor FUNDAMENT
- JBScreen Thermofluor SPECIFIC
- α-Chymotrypsinogen A (0.25 µg/µl final concentration)
- T4 dNMP Kinase (0.1 µg/µl final concentration)
- SYPRO® Orange (1:1000 for α-Chymotrypsinogen A, 1:1250 for T4 dNMP Kinase)

The different reaction mixtures were prepared according to the following tables.

Table 1: Sample/ Control preparation for 25 µl reaction volume

	Sample Chymotryp-sinogen	Sample Kinase	Control Chymotryp-sinogen	Control Kinase
Screening Solution	15.00 µl	15.00 µl	-	-
α-Chymotrypsinogen A	2.50 µl	-	2.50 µl	-
T4 dNMP Kinase	-	2.50 µl	-	2.50 µl
SYPRO® Orange	1.25µl	1.00 µl	1.25 µl	1.00 µl
H_2O	6.25µl	6.50 µl	21.25 µl	21.50 µl
Final Volume	25.00 µl	25.00 µl	25.00 µl	25.00 µl

Table 2: Sample/ Control preparation for 5 µl reaction volume

	Sample Chymotryp-sinogen	Sample Kinase	Control Chymotryp-sinogen	Control Kinase
Screening Solution	3.00 µl	3.00 µl	-	-
α-Chymotrypsinogen A	0.50 µl	-	0.50 µl	-
T4 dNMP Kinase	-	0.50 µl	-	0.50 µl
SYPRO® Orange	0.25 µl	0.20 µl	0.25 µl	0.20 µl
H_2O	1.25 µl	1.30 µl	4.25 µl	4.30 µl
Final Volume	5.00 µl	5.00 µl	5.00 µl	5.00 µl

Instrument

- qTOWER³ with color module Protein 1 (490 nm / 580 nm)

PCR time and temperature protocol

The qTOWER³ was programmed according to the following protocol.

Table 3: Time and temperature protocol

Step	Cycle	Profile	Temperature	Holding time	Ramp rate
1	1	Temp. equilibration*	25 °C	10 s	max.
2	1	Melting curve*	25 – 90 °C with equilibration of 10 s and ΔT = 1 °C		

*** Data acquisition:** Color Module Protein 1 (490 – 580 nm) with Gain 5 for 25µl and Gain 8 for 5µl

Layout

JBScreen Thermofluor is designed to systematically screen for the most important protein stabilizing parameters such as pH, ionic strength and specific ions. The application of so called “Super Buffers” allows an independent pH screening, by keeping the chemical environment of the buffer solution constant over the pH range from 4.0 to 10.0.

JBScreen Thermofluor FUNDAMENT identifies the optimum pH and ionic strength that mostly stabilizes the target protein. JBScreen Thermofluor SPECIFIC further analyses specific metal ions that affect protein thermostability. The screens are supplied in a deep well block containing 93 screening solutions each.

	1	2	3	4	5	6	7	8	9	10	11	12
A	CP + Dye CB-1	CP + Dye CB-1	CP + Dye CB-1	CP + Dye CB-2	CP + Dye CB-2	CP + Dye CB-2	CP + Dye CB-3	CP + Dye CB-3	CP + Dye CB-3	TP + Dye Original Buffer	TP + Dye Original Buffer	TP + Dye Original Buffer
B	4.0	4.0 50 mM NaCl	4.0 125 mM NaCl	4.0 250 mM NaCl	4.0 500 mM NaCl	4.0 1 M NaCl	4.0	4.0 50 mM NaCl	4.0 125 mM NaCl	4.0 250 mM NaCl	4.0 500 mM NaCl	4.0 1 M NaCl
C	5.0	5.0 50 mM NaCl	5.0 125 mM NaCl	5.0 250 mM NaCl	5.0 500 mM NaCl	5.0 1 M NaCl	5.0	5.0 50 mM NaCl	5.0 125 mM NaCl	5.0 250 mM NaCl	5.0 500 mM NaCl	5.0 1 M NaCl
D	6.0	6.0 50 mM NaCl	6.0 125 mM NaCl	6.0 250 mM NaCl	6.0 500 mM NaCl	6.0 1 M NaCl	6.0	6.0 50 mM NaCl	6.0 125 mM NaCl	6.0 250 mM NaCl	6.0 500 mM NaCl	6.0 1 M NaCl
E	7.0	7.0 50 mM NaCl	7.0 125 mM NaCl	7.0 250 mM NaCl	7.0 500 mM NaCl	7.0 1 M NaCl	7.0	7.0 50 mM NaCl	7.0 125 mM NaCl	7.0 250 mM NaCl	7.0 500 mM NaCl	7.0 1 M NaCl
F	8.0	8.0 50 mM NaCl	8.0 125 mM NaCl	8.0 250 mM NaCl	8.0 500 mM NaCl	8.0 1 M NaCl	8.0	8.0 50 mM NaCl	8.0 125 mM NaCl	8.0 250 mM NaCl	8.0 500 mM NaCl	8.0 1 M NaCl
G	9.0	9.0 50 mM NaCl	9.0 125 mM NaCl	9.0 250 mM NaCl	9.0 500 mM NaCl	9.0 1 M NaCl	9.0	9.0 50 mM NaCl	9.0 125 mM NaCl	9.0 250 mM NaCl	9.0 500 mM NaCl	9.0 1 M NaCl
H	10.0	10.0 50 mM NaCl	10.0 125 mM NaCl	10.0 250 mM NaCl	10.0 500 mM NaCl	10.0 1 M NaCl	10.0	10.0 50 mM NaCl	10.0 125 mM NaCl	10.0 250 mM NaCl	10.0 500 mM NaCl	10.0 1 M NaCl

pH

100 mM Super Buffer 1 | 100 mM Super Buffer 2

Ionic Strength | Ionic Strength

Figure 1: Layout of the JBScreen Thermofluor FUNDAMENT

	1	2	3	4	5	6	7	8	9	10	11	12
A	CP + Dye CB-1	CP + Dye CB-1	CP + Dye CB-1	CP + Dye CB-2	CP + Dye CB-2	CP + Dye CB-2	CP + Dye CB-3	CP + Dye CB-3	CP + Dye CB-3	TP + Dye Original Buffer	TP + Dye Original Buffer	TP + Dye Original Buffer
B	4.0	4.0	4.0 20 mM MgSO4	4.0 10 mM FeCl3	4.0 10 mM ZnCl2	4.0 10 mM MnCl2	4.0	4.0	4.0 20 mM MgSO4	4.0 20 mM CaCl2	4.0 10 mM ZnCl2	4.0 10 mM MnCl2
C	5.0	5.0	5.0 20 mM MgSO4	5.0 10 mM FeCl3	5.0 10 mM ZnCl2	5.0 10 mM MnCl2	5.0	5.0	5.0 20 mM MgSO4	5.0 20 mM CaCl2	5.0 10 mM ZnCl2	5.0 10 mM MnCl2
D	6.0	6.0	6.0 20 mM MgSO4	6.0 10 mM FeCl3	6.0 10 mM ZnCl2	6.0 10 mM MnCl2	6.0	6.0	6.0 20 mM MgSO4	6.0 20 mM CaCl2	6.0 10 mM ZnCl2	6.0 10 mM MnCl2
E	7.0	7.0	7.0 20 mM MgSO4	7.0 10 mM FeCl3	7.0 20 mM LiCl	7.0 20 mM KCl	7.0	7.0	7.0 20 mM MgSO4	7.0 20 mM CaCl2	7.0 20 mM LiCl	7.0 20 mM KCl
F	8.0	8.0	8.0 20 mM MgSO4	8.0 10 mM FeCl3	8.0 20 mM LiCl	8.0 20 mM KCl	8.0	8.0	8.0 20 mM MgSO4	8.0 20 mM CaCl2	8.0 20 mM LiCl	8.0 20 mM KCl
G	9.0	9.0	9.0 20 mM MgSO4	9.0 10 mM FeCl3	9.0 20 mM LiCl	9.0 20 mM KCl	9.0	9.0	9.0 20 mM MgSO4	9.0 20 mM CaCl2	9.0 20 mM LiCl	9.0 20 mM KCl
H	10.0	10.0	10.0 20 mM MgSO4	10.0 10 mM FeCl3	10.0 20 mM LiCl	10.0 20 mM KCl	10.0	10.0	10.0 20 mM MgSO4	10.0 20 mM CaCl2	10.0 20 mM LiCl	10.0 20 mM KCl

pH

100 mM Super Buffer 1 | 100 mM Super Buffer 2

150 mM NaCl | 150 mM NaCl

Figure 2: Layout of the JBScreen Thermofluor SPECIFIC

Results and Discussion

The following figures show a comparison of thermal shift assays generated in the qTOWER³ with 25 µl and 5 µl reaction volume. The results show higher melting temperatures for 5 µl compared to 25 µl but their overall behaviour is equivalent and reproducible within the volume range. Therefore, stabilizing conditions can also be extracted from the 5 µl reaction volumes.

JBScreen Thermofluor FUNDAMENT with α-Chymotrypsinogen A (Figure 3)

The most stabilizing conditions for α-Chymotrypsinogen A were found in the pH range from 5-6. The melting temperature increased by 7°C compared to the initial buffer conditions (K, first 3 bars).
At pH>6, the ionic strength is directly proportional to the protein thermostability.

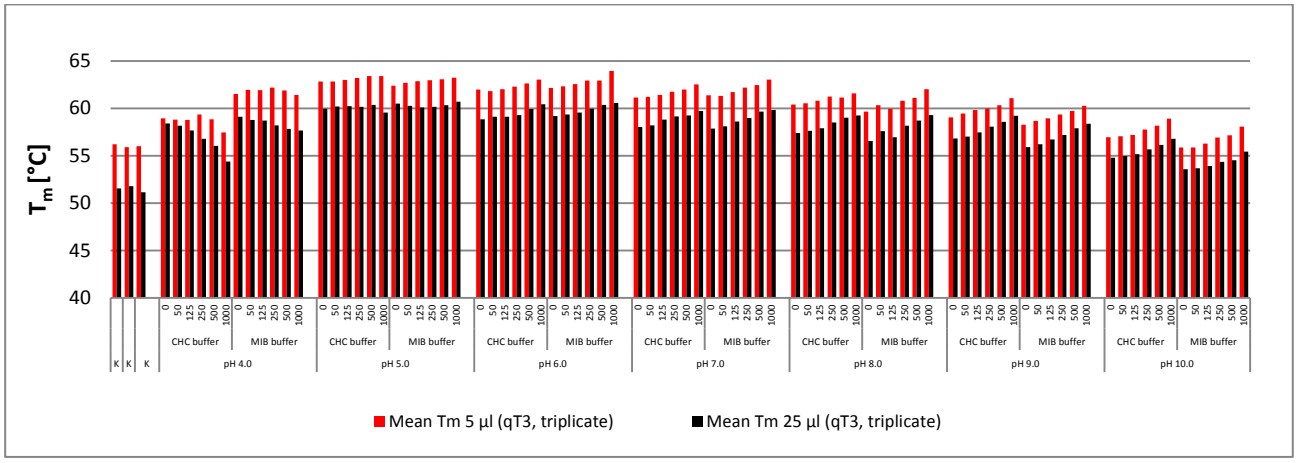

Figure 3: Effect of pH and NaCl concentration on the thermostability of α-Chymotrypsinogen A in 5 µl and 25 µl reaction volume set up with JBScreen Thermofluor FUNDAMENT.

JBScreen Thermofluor FUNDAMENT with T4 dNMP Kinase (Figure 4)

T4 dNMP Kinase is most stable in pH 6-8, the melting temperature significantly increased compared to the initial buffer conditions. Again, ionic strength is correlated with protein thermostability, especially at higher pH values.

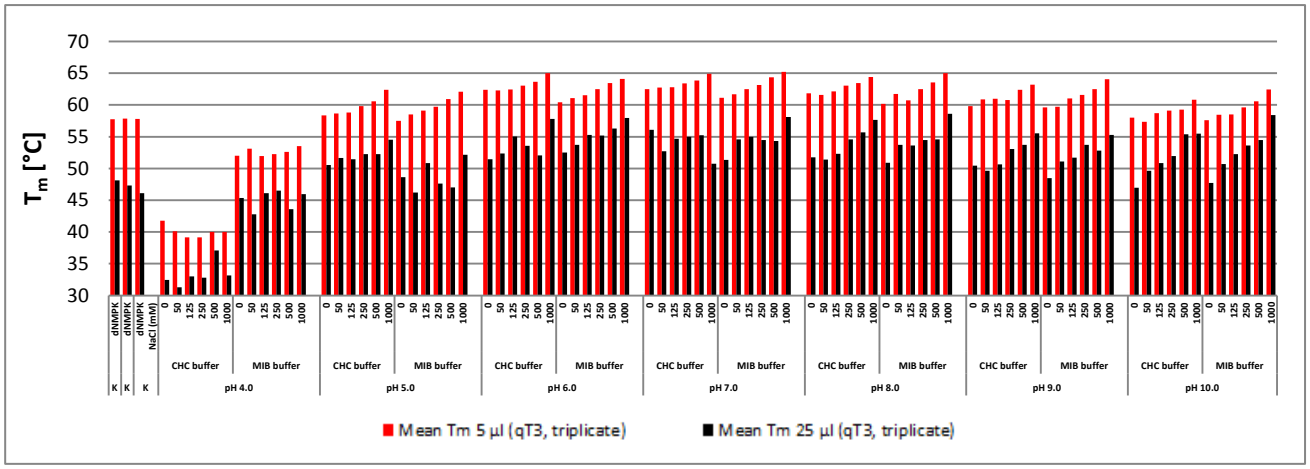

Figure 4: Effect of pH and NaCl concentration on the thermostability of T4 dNMP Kinase in 5 µl and 25 µl reaction volume set up with JBScreen Thermofluor FUNDAMENT.

JBScreen Thermofluor SPECIFIC with α-Chymotrypsinogen A (Figure 5)

α-Chymotrypsinogen A has shown increased thermostability in all screen formulations when compared to its initial buffer condition (K, first 3 bars). Above pH 5.0 a significant stabilization is seen in the presence of Ca^{2+} - a well-known ligand of α-Chymotrypsinogen A. Interestingly, similar effects are obtained in the presence of the metal ion Fe^{3+} in the higher pH ranges.

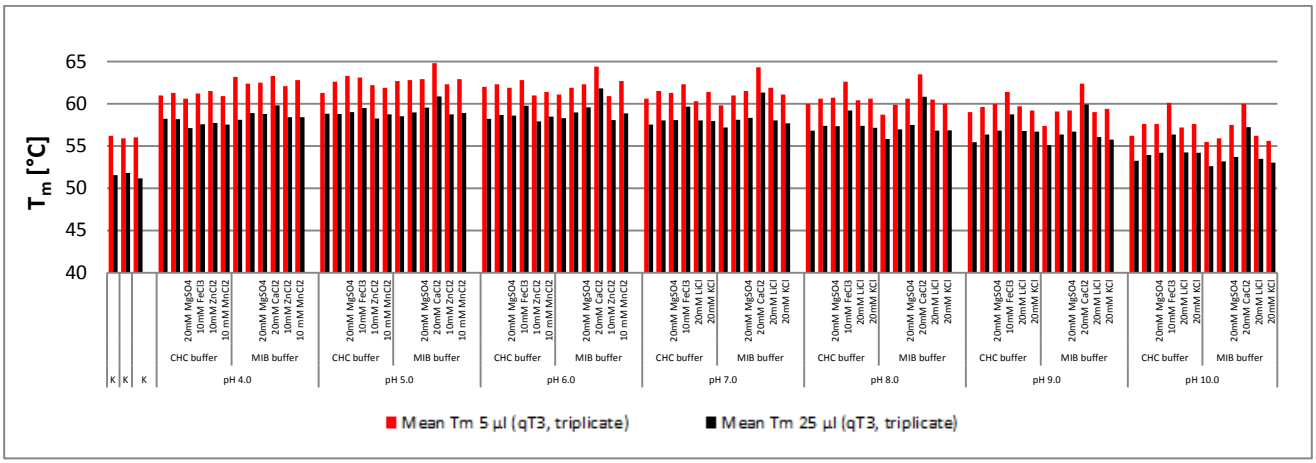

Figure 5: Effect of metal ions as a function of pH on the thermostability of α-Chymotrypsinogen in 5 µl and 25 µl reaction volume set up with JBScreen Thermofluor SPECIFIC.

JBScreen Thermofluor SPECIFIC with T4 dNMP Kinase (Figure 6)

In the case of T4 dNMP Kinase no relevant effect could be identified in the presence of metal ions of different valencies.

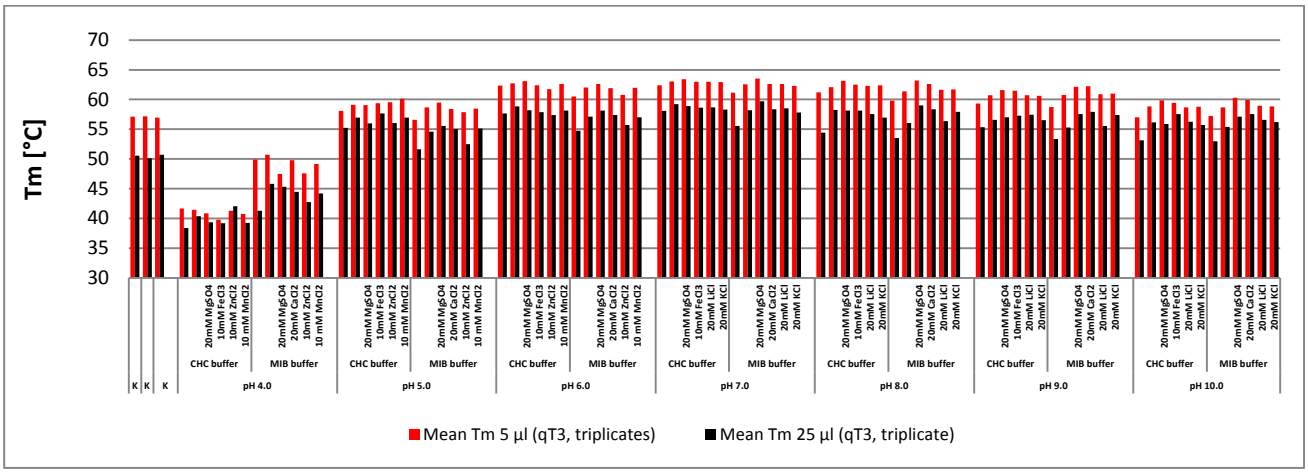

Figure 6: Effect of metal ions as a function of pH on the thermostability of T4 dNMP Kinase in 5 µl and 25 µl reaction volume set up with JBScreen Thermofluor SPECIFIC.

Summary

The results show, that JBScreen Thermofluor FUNDAMENT and JBScreen Thermofluor SPECIFIC is suitable for stability screening of different proteins using Analytik Jena's real-time PCR cycler qTOWER³. Even when the reaction volume is reduced by 80%, the achieved outcome for the stability of the examined proteins is congruent without any loss of performance and meaningfulness.

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