02/2015

Prometheus NT.48

Product Information

Prometheus Instruments
for nanoDSF

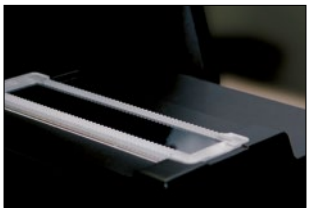
www.nanotemper-technologies.com

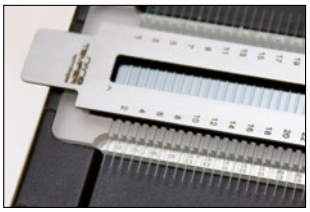
Prometheus NT.48

NanoTemper Technologies offers nanoDSF technology with the Prometheus Series. nanoDSF is the method of choice for easy, rapid and accurate analysis of protein folding and stability, with applications in protein engineering, formulation development and quality control.

Enjoy the benefits of nanoDSF:

- See more transitions with high-resolution data
- Get faster results with lower sample quantities
- Measure within a broad concentration range, from 5 µg/ml to 150 mg/ml
- Avoid labeling, ensuring buffer & detergent independent measurements





Prometheus NT.48

nanoDSF

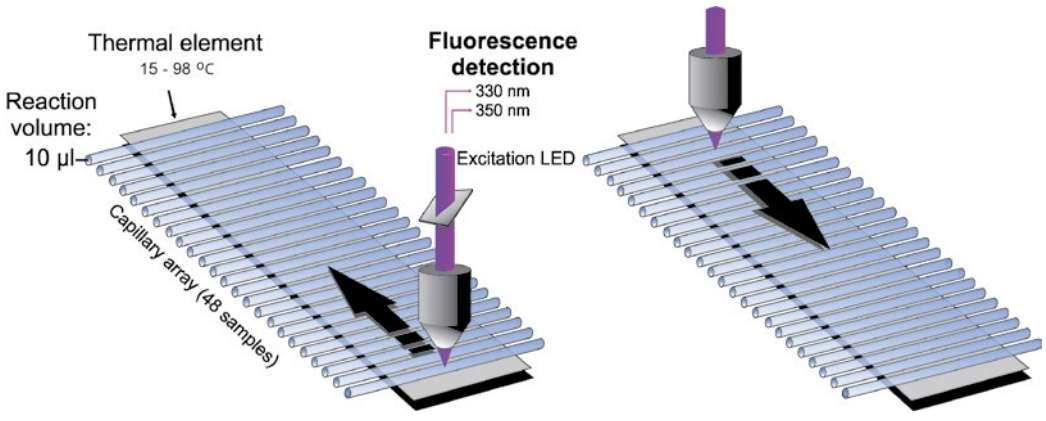
A technology by NanoTemper

nanoDSF is an advanced Differential Scanning Fluorimetry technology. It detects smallest changes in the fluorescence of tryptophan present in virtually all proteins.

The fluorescence of tryptophans in a protein is strongly dependent on close surroundings. By following changes in fluorescence, chemical and thermal stability can be assessed in a truly label-free fashion.

The dual-UV technology by NanoTemper allows for rapid fluorescence detection, providing an unmatched scanning speed and data point density. This yields an ultra-high resolution unfolding curves which allow for detection of even minute unfolding signals.

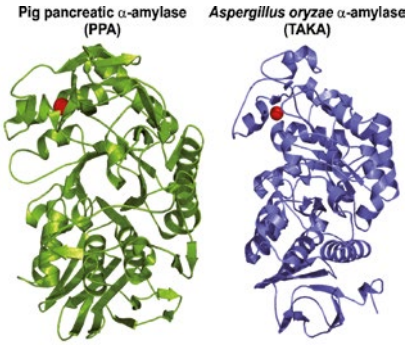
Furthermore, since no secondary reporter fluorophores are required as in conventional DSF, protein solutions can be analyzed independent of buffer compositions, and over a concentration range of 150 mg/ml down to 5 µg/ml. This allows for the analysis of detergent-solubilized membrane proteins, as well as for highly concentrated antibody formulations.

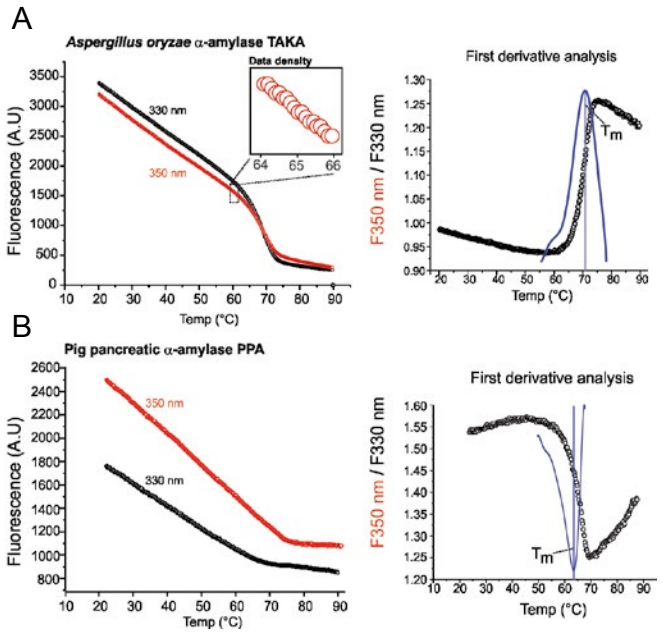


Technical Details

	Prometheus NT.48
Samples per run	48 samples
Fluorescence detection	330 / 350 nm
Labeling required	No labeling, no dye
Concentration of fluorescent molecule	5 µg/ml to > 150 mg/ml
Molecular weight range (Da)	10^1 - 10^7
Volume per measurement	10 µl
Temperature controlled	15 °C - 98 °C
Heating rate	0.1 - 5 °C/min
Precision	± 0.2 °C
Biophysical parameters	Denaturation midpoints Tm and Cm
Tryptophan fluorescence required	Yes
Measurements in detergents	Yes
Time for experiment & analysis	Minutes - hours
Maintenance required	No

Results: Analyzing the Thermal Stability of Amylases





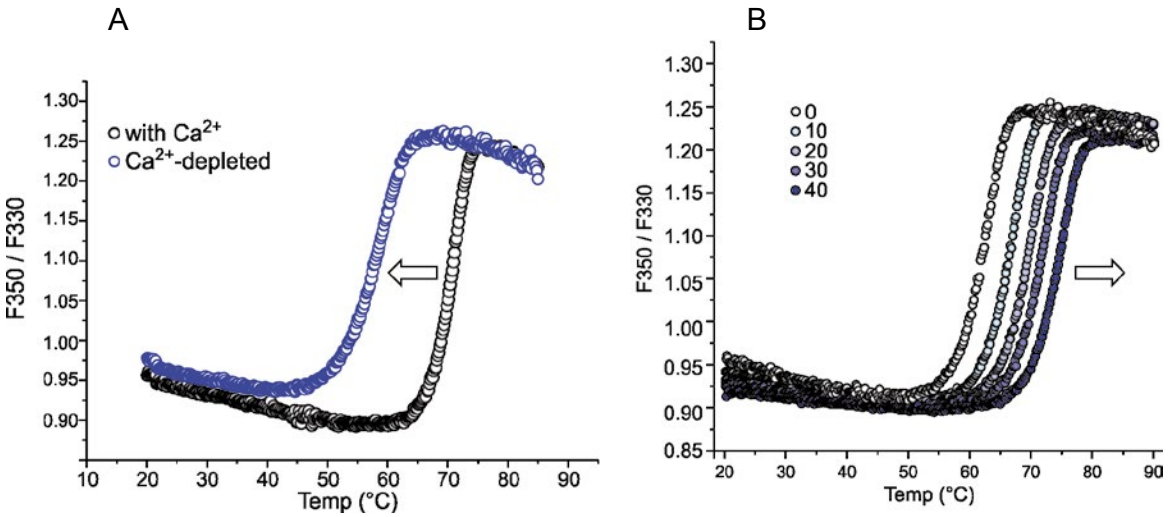
Crystal structure and thermal stability of two Amylase isoforms.
(A) Two wavelenghts, 330 and 350nm are recorded. The ratio of the two wavelengths is plotted against the temperature. The first derivative can be deduced to determine the Tm.
(B) The 350/330nm ratio typically yields well-defined transitions, even if the single wavelengths do not exhibit a clear unfolding transition.

Protein denaturation curves are used to derive important stability parameters. The thermal stability of a given protein is typically described by the thermal unfolding transition midpoint Tm (°C), at which half of the protein population is unfolded.

Tm can be calculated from the changes in tryptophan fluorescence intensity, or from the ratio of tryptophan emission at 330 and 350 nm, which describes the shift of tryptophan emission upon unfolding.

Typically, the 350/330 nm ratio yields data with well-defined transitions upon protein unfolding, whereas the single wavelength detection does not always allow one to derive the Tm. Thus, the dual wavelength system of the Prometheus NT.48 provides a sensitive label-free detection for unfolding processes.

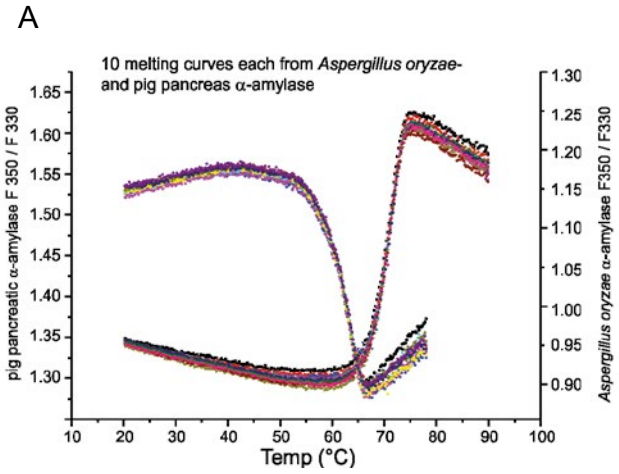
Results: Amylase Stability in Different Buffer Conditions



Stability of *Aspergillus oryzae* Amylase TAKA in different buffer conditions

(A) Removal of Ca^{2+} ions results in a marked destabilization of amylase, as indicated by the shift of Tm towards lower values.

(B) In order to find optimal conditions for an increased thermal stability of amylase, its thermal unfolding was monitored under 5 different sucrose concentrations.



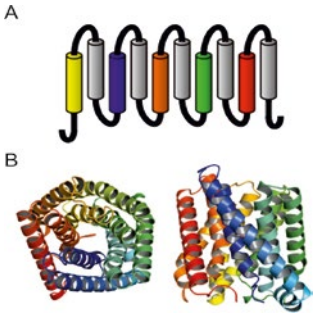
Average Tm values of α-amylase from different species:

Protein	Tm (°C)	Literaure Tm value (°C)
Aspergillus oryzae	71.3 +/- 0.20*	70
pig pancreas	63.6 +/- 0.17*	65

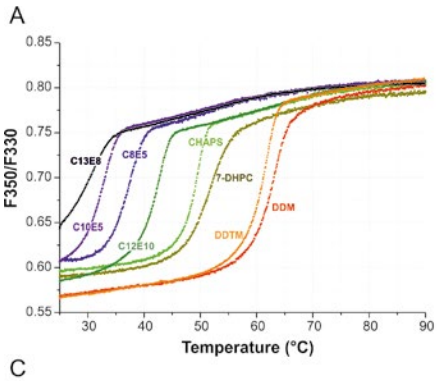
* SD from 10 capillaries, Tm determined by first derivative analysis

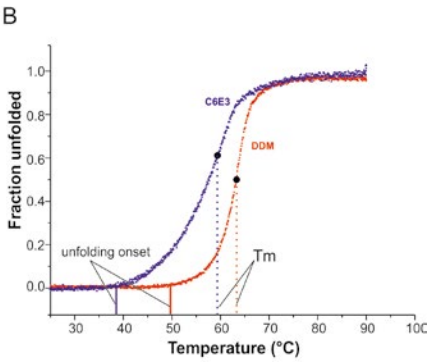
Precision and reproducibility of Prometheus NT.48 unfolding data.
(A) The plots represent an overlay of 10 independently recorded melting curves of PPA and TAKA, respectively.
(B) Determination of Tm for both proteins displays a small standard deviation between experiments (≤ 0.2 °C) and a good correlation with published results (Fitter, J., Structural and dynamical features contributing to thermostability in alpha-amylases. Cell Mol Life Sci, 2005. 62(17): p. 1925-37.)

Results: Detergent Screen for a Membrane Protein



Structure of HiTehA.
A) Schematic representation of the domain organization of SLAC1-like transmembrane proteins. B) Crystal structure of HiTehA showing the quasi-five-fold symmetrical arrangement of transmembrane helices around the central pore.





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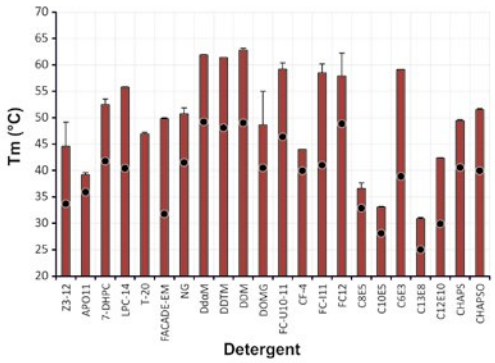
Detergent	Tm	unfolding onset
Z3-12	44.585	33.692
FC12	57.88	48.837
7-DHPC	52.475	41.773
CHAPS	49.415	40.562
APO11	39.205	35.909
C8E5	36.565	32.858
C12E10	42.33	29.897
NG	50.72	41.494
DDM	62.79	41.962
DOMG	48.635	40.491
FC-U10-11	59.185	33.998
LPC-14	55.755	40.393
T-20	46.905	n.a.
C10E5	33.06	28.089
C13E8	30.865	25.026
FACADE-EM	49.805	31.751
DdαM	61.885	49.196
DDTM	61.37	48.078
CF-4	43.94	39.951
FC-I11	58.485	41.022
CHAPSO	51.51	39.951
C6E3	59.11	38.887



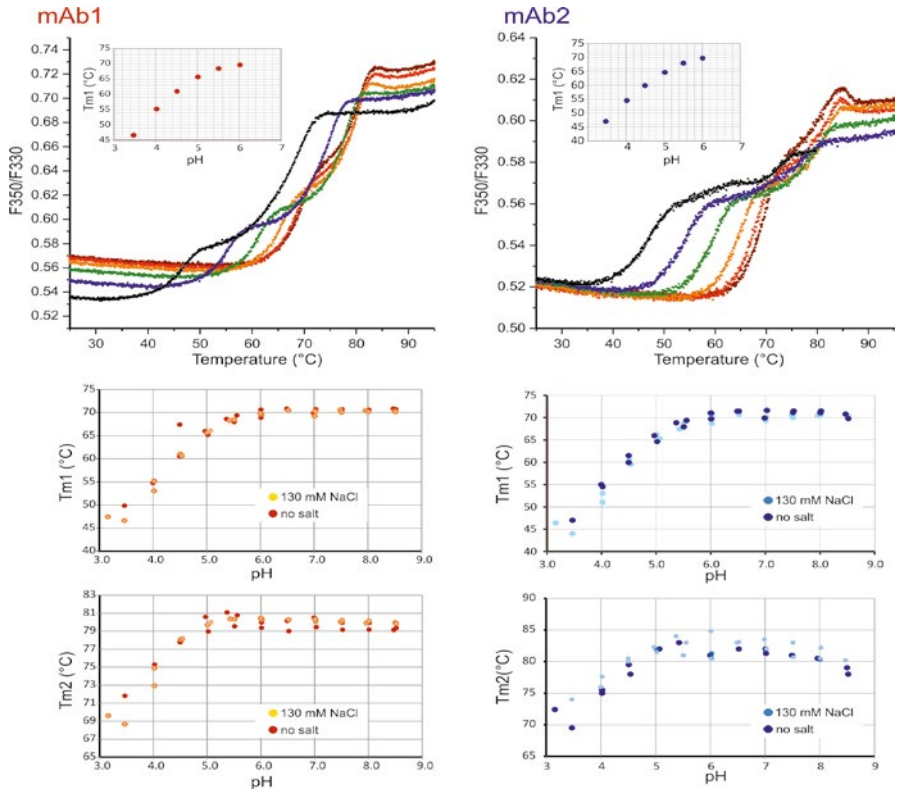
Detergent screen of HiTehA.
A) Representative thermal unfolding curves of HiTehA in 50 mM Tris HCl pH 8.0, 200 mM NaCl and the respective detergents. B) Example for different unfolding onset temperatures in presence of C6E3 and DDM.
C) Summary of Tm values (red bars, right) and unfolding onset temperatures (black dots, right) in presence of different detergents.

Our results show that monitoring of tryptophan fluorescence emission shifts during thermal unfolding, using the Prometheus NT.48, is a perfect approach to determine the optimal conditions for membrane protein purification and storage.

Notably, micelle formation, autofluorescence or other secondary effects which are common for detergents, and which often interfere with biophysical characterization of membrane protein stability, do not impede measurements employing the Prometheus NT.48.

Moreover, the high precision and sensitivity of the instrument allows for detection of very low protein concentrations in the range of a few µg/ml in as little as 10 µl of sample, thus protein consumption is very small compared to other methods.

Results: Optimum Buffer Conditions for Antibody Formulations



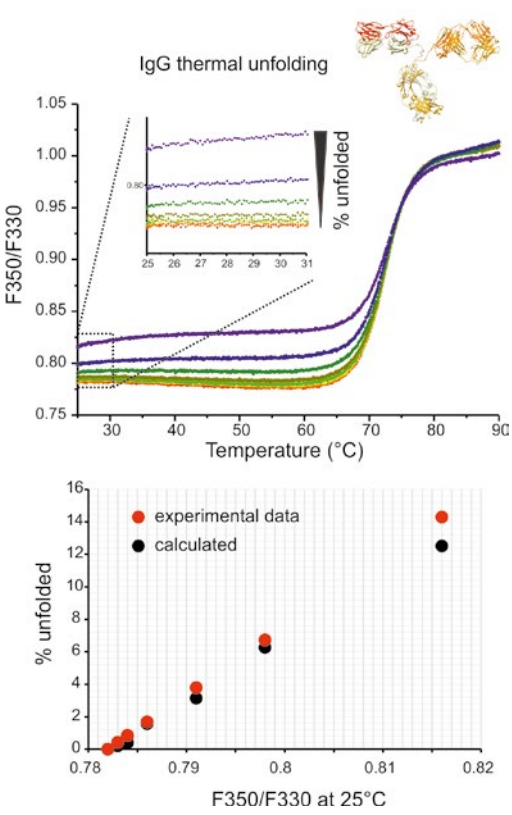
Thermal unfolding curves and unfolding transition midpoints of antibodies mAb1 and mAb2.
(A) Thermal unfolding curves of mAb1 and mAb2 in presence of 25 mM Na-Citrate at different pH values. Insets show the pH-dependence of the first unfolding transition midpoint (Tm1).
(B) Dependence of Tm1 and Tm2 on the pH of the buffer for all tested conditions.

Our data demonstrate that the Prometheus NT.48 delivers high quality thermal unfolding data for antibody buffer screening campaigns. In summary, 45 different buffer conditions were tested on 2 antibodies, and 90 thermal unfolding curves have been recorded and analyzed within less than 2.5 hours.

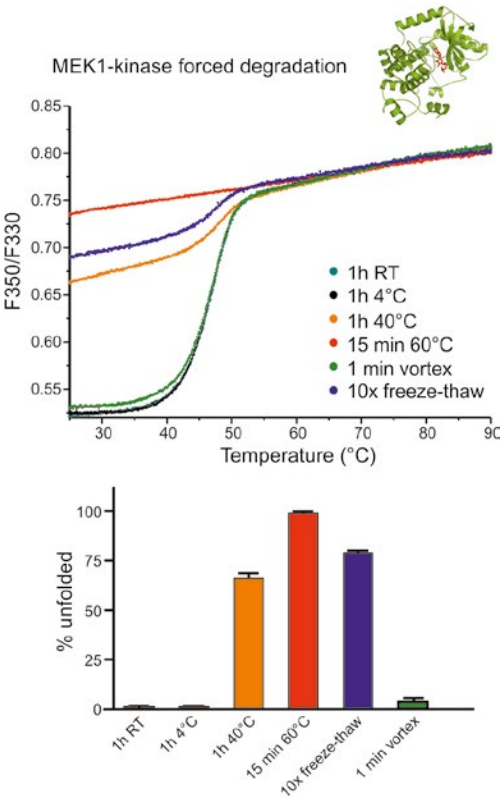
The large dynamic range of the Prometheus NT.48 allows for analyzing thermal unfolding in solutions containing antibody concentrations between 150 mg/ml down to few µg/ml. Thus, it can be utilized for both stability screening during the early phases of antibody development, where only small amounts of protein are available as well as for final formulation screenings campaigns in highly concentrated samples.

The high-resolution thermal unfolding curves also enable separate and rapid assessment of the effects of buffers, excipients or even covalent modifications on the stability of distinct antibody domains.

Results: Quality Control



Establishing a protein unfolding standard.
Unfolded IgG at different concentrations was mixed with folded IgG and subject to thermal unfolding. The percentage of unfolded IgG in the solution was quantified based on the F350/F330 ratio measured at 25 °C.



Forced-degradation stress-test on MEK1
MEk1 protein was subject to the indicated stresses, and the fraction of unfolded protein was calculated based on the F350/F330 ratio at 25 °C. Error bars are s.d. from three measurements.

The presented data demonstrate that the Prometheus NT.48 can be employed to quickly detect and quantify unfolded proteins for quality control purposes with unmatched speed, at the same time offering unique ease of use.

The presented quality control experiments can be performed by filling capillaries directly from stock solutions without laborious sample preparation or loading. F350/F330 values of up to 48 samples are then recorded in parallel using a one-button routine, providing stability data within seconds.

Customer Statements

Prof. Dr. Thomas Müller, University of Wuerzburg, Germany

Commonly used methods to determine protein stability suffer from various drawbacks requiring either rather large amounts (DSC), specific conditions (CD), or otherwise cannot be applied to all biomolecules species (ThermoFlour). In particular very few methods exist for membrane proteins, as the requirement of detergent-containing buffers very often impedes the use of CD spectroscopy (due to strong light absorption by the detergent) and the application of the usually preferred ThermoFluor methodology (due to binding of the dye to the detergent micelles). The capabilities of the new Prometheus NT.48 in measuring thermal unfolding now allows us to quickly determine the ideal buffer conditions and the detergent best suited for crystallization trials. Fast measurements, very low material consumption, label-free capabilities, and low background noise make the Prometheus NT.48 the best current solution for screening membrane protein buffer conditions.

Dr. Neil Ferguson, University College Dublin, Ireland

“Over the years, my co-workers and I have measured the stability of many tens of thousands of proteins (or mutants thereof). Typically, these take the form of chemical denaturant titrations or thermal denaturation assays. Whilst the latter has proved possible to automate via different strategies, chemical denaturant titrations have historically been slow, required user skill and continual intervention to achieve good results and consumed lots of protein. This has led to denaturant titrations being a niche, primarily academic, technique.
However, the NanoTemper Prometheus changes this, as it allows very rapid, low protein consumption determination of protein stability using chemical and/or thermal denaturation. Thus, the Prometheus is an ideal workhorse for academic research involving large-scale protein engineering or stability measurements. ”

Dr. Thomas Schubert, 2bind GmbH

“2bind offers service for biomolecular interaction studies using MicroScale Thermophoresis. Our customers provide samples at different stages in their projects, sometimes with only little knowledge about the stability of different constructs and suitable buffer conditions.
The Prometheus NT.48 now allows us to quickly test the stability of proteins in different buffer conditions and even in the presence of detergents. This enables us to also measure membrane proteins in close to native conditions, which is not possible with the conventional DSF method. The unmatched resolution due to the high density of data points are very beneficial for antibody engineering projects since multiple transitions can be identified and determined with high accuracy and reproducibility.
We also appreciate that the instrument is very robust and absolutely maintenance-free.”

Selected Publications

1. Amaning K, Lowinski M, Vallee F, Steier V, Marcireau C, Ugolini A, Delorme C, Foucalt F, McCort G, Derimay N, Andouche C, Vougier S, Llopart S, Halland N, Rak A (2013) The use of virtual screening and differential scanning fluorimetry for the rapid identification of fragments active against MEK1. Bioorganic & medicinal chemistry letters 23: 3620-3626
2. Alexander, C. G., Wanner, R., Johnson, C. M., Breitsprecher, D., Winter, G., Duhr, S., Baaske, P., and Ferguson, N. (2014) Novel microscale approaches for easy, rapid determination of protein stability in academic and commercial settings. Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics 1844, 2241-2250
3. Breitsprecher D, Linke P, Roth HM, Rak A (2014) Case Study on Automated Screening Project of a Fragment Library against MEK1 kinase. Application Note NT_MO-021
4. Martin, L., Maschberger, M., and Breitsprecher, D. (2015) Thermal Stability Buffer Screening of Therapeutic Antibodies. Application Note NT-PR-003
5. Martin, L., Schwarz, S., and Breitsprecher, D. (2014) Analyzing Thermal Unfolding of Proteins: The Prometheus NT.48. Application Note NT-PR-001
6. Maschberger, M., and Breitsprecher, D. (2015) Rapid Quantification of Unfolded Proteins for Quality Control and Optimization of Storage Conditions. Application Note NT-PR-004
7. Maschberger, M., Hüttl, S., Müller, T. D., and Breitsprecher, D. (2015) Detergent Screen for solubilized membrane proteins – Case study on the SLAC-protein HiTehA from Haemophilus influenzae. Application Note NT-PR-002
8. Oyarzabal J, Zarich N, Albarran MI, Palacios I, Urbano-Cuadrado M, Mateos G, Reymundo I, Rabal O, Salgado A, Corrionero A, Fominaya J, Pastor J, Bischoff JR (2010) Discovery of mitogen-activated protein kinase-interacting kinase 1 inhibitors by a comprehensive fragment-oriented virtual screening approach. Journal of medicinal chemistry 53: 6618-6628
9. Raynal B, Lenormand P, Baron B, Hoos S, England P (2014) Quality assessment and optimization of purified protein samples: why and how? Microbial cell factories 13: 986

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Europe / International

NanoTemper Technologies GmbH
Flößergasse 4
81369 München
Germany

Phone +49 (0)89 4522895 0
Fax +49 (0)89 4522895 60

info@nanotemper-technologies.com

South America

NanoTemper Technologies GmbH
Center Alameda Santos
Alameda Santos, 200
São Paulo -SP 01418-000, Brazil

Phone: +56 (2) 2581-4664
or +55 (11) 3587-1408

info@nanotemper-technologies.com

USA / Canada

NanoTemper Technologies, Inc.
395 Oyster Point Blvd. Suite 135
South San Francisco,
CA 94080, USA

Phone + 1 650 763 1658
Fax + 1 650 350 4390

info@nanotemper-technologies.com