User Manual
Prometheus Series Instruments

Content

Safety Considerations

To ensure operation safety, this instrument must be operated correctly. Carefully read the following explanations to fully understand all safety precautions in this manual before operating the instrument. Please take a moment to understand what the signal words **WARNING!, CAUTION** and **NOTE** mean in this manual.

Safety symbols

WARNING! A **WARNING!** indicates a potentially hazardous situation which, if not avoided, may result in serious injury or even death.

CAUTION A **CAUTION** indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. **CAUTION** may also be used to alert against damaging the equipment or the instrument.

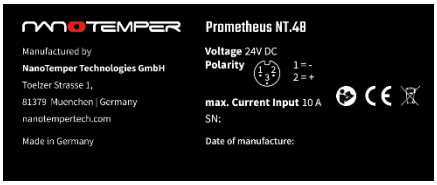
Do not proceed beyond a **WARNING!** or **CAUTION** notice until you understand the hazardous conditions and have taken appropriate steps.

NOTE A **NOTE** provides additional information to help the operator achieve optimal instrument and assay performance.

Read user manual label. This label indicates that you have to read the user manual before using the instrument. This label is positioned at the back of the device.

Warning symbol. This symbol indicates a surface that can heat up and cause burn injuries. This warning label is positioned on the sample tray.

Warning symbol. This symbol indicates a possible risk for hand injuries by crushing and sharp edges. This warning label is positioned on the sample tray.



Identification label. This label is positioned at the rear panel of the device.

WARNING! Operate the Prometheus Series instrument only with the provided external power supply. Only use the provided cables and plugs. Failure to comply may result in a risk of electric shock and fire.

CAUTION The Prometheus Series instrument has to be installed in a way that does not hinder access to the external power supply and its power plug.

CAUTION Connect the Prometheus Series instrument power supply in a way that avoids tripping hazards.

CAUTION Do not use extension cords. Damaged cords, plugs or cables need to be replaced immediately. Failure to comply may result in a risk of personal injury or damage to the instrument.

WARNING! Do not operate the Prometheus Series instrument with substances or under conditions that pose a risk of explosion, implosion or release of gases. Do not use the instrument with hazardous or infectious substances.

CAUTION Use only aqueous samples for analysis in the instrument.

CAUTION The Prometheus Series instrument weighs approx. 30 kg. Two people are required for transport. Moving the instrument alone entails a risk of personal injury or damage to the instrument.

CAUTION Do not open the instrument manually or anywhere other than the sample loading drawer. Opening entails a risk of personal injury or damage to the instrument and may only be done by NanoTemper Technologies staff.

CAUTION Only NanoTemper Technologies staff may service the instrument. Turn off the power switch and unplug the power cord before servicing the instrument, unless instructed otherwise.

CAUTION The instrument contains a UV-LED. The UV-LED emits invisible ultraviolet radiation (UVB radiation) when in operation, which may be harmful to eyes and skin, even at brief periods of exposure. Do not look directly into the UV-LED during operation. If used as intended, the instrument emits no UV radiation.

CAUTION Mechanical moving parts within the instrument can pinch or injure your hands or fingers. Do not touch or open the instrument while parts are moving.

CAUTION The display pane is made of glass. Broken glass can injure your hands or fingers.

CAUTION The sharp edges of the Prometheus NT.Plex chipholder thermal element pose a risk of injury to hands and fingers.

CAUTION The instrument contains a temperature regulator to control the sample temperature. Some accessible parts of the instrument can reach temperatures of up to 60 °C. Don’t touch the temperature controlled parts of the instrument when the temperature controller is set to high temperatures.

CAUTION Do not use the instrument at ambient temperatures below 15 °C.

CAUTION Use the instrument only at noncondensing conditions (0–80 % humidity, 15-30 °C). At very high humidity levels, even normal operating temperatures may result in condensation and corrosion.

CAUTION Turn off the instrument when not in use.

CAUTION Do not use ethanol or other types of organic solvents to clean the instrument as they may remove the instrument paint.

CAUTION Remove loose parts (capillaries and capillary lid) before transport as they may damage the measurement optics

Regulatory Statement

The following safety and electromagnetic standards were considered:

- IEC 61010-1:2010/AMD1:2016 Safety requirements for electrical equipment for measurement, control and laboratory use. Part 1 General Requirements
- IEC 61010-2-010:2019 Safety requirements for electrical equipment for measurement, control and laboratory use. Part 2-010: Particular requirements for laboratory equipment for the heating of materials.
- IEC 61010-2-081:2019 Safety requirements for electrical equipment for measurement, control and laboratory use. Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes.
- IEC 61326-1:2006 EMC, Electrical equipment for measurement, control and laboratory use – EMC requirements.
- IEC 61000-3-2:2006 EMC, Limits for harmonic current emissions (equipment input current up to and including 16A per phase).
- IEC 61000-3-3:2008 EMC, Limits

Technical Specifications

Input of external power supply: 90–264 VAC ± 10 % 47–63 Hz, 230 VA max
Output of external power supply: 24 VDC, 10 A max

Electrical input to Prometheus Series instrument: 24 VDC, 10 A

Pollution degree: 2

Environmental:
Operating temperature 15 – 30 °C (indoor use only)
Humidity 0–80 %, noncondensing
Operating altitude max 2,000 m

Prometheus Series instrument dimensions:
Width 35 cm (13.8”)
Height 51 cm (20.1”)
Depth 52 cm (20.5”)
Weight 30 kg (66 lbs) net

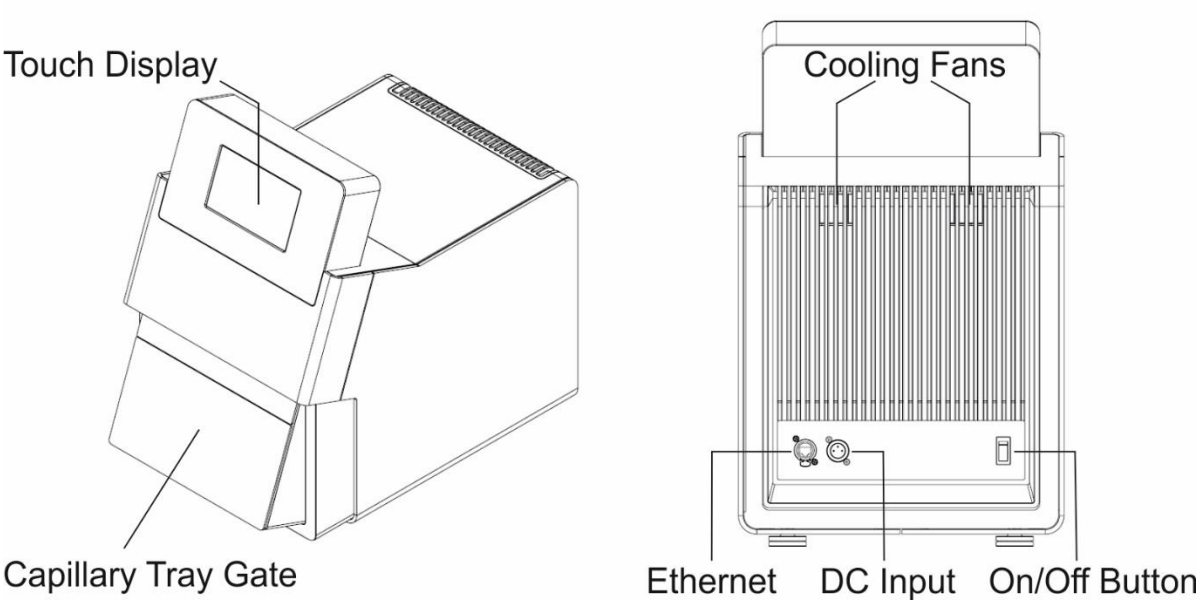
Power supply dimensions:
Width 21 cm (8.3”)
Height 9 cm (3.5”)
Depth 3 cm (1.1”)
Weight 0.5 kg (1.1 lbs) net max

Temperature control:
Range: 15 °C – 95 °C (at 25 °C)
Optional High Temperature Upgrade: 15 °C – 110 °C (at 25 °C)
Accuracy: +/- 0.1 °C

Noise level of Prometheus Series instrument: max. 64 dB(A)

Connections

All ingoing and outgoing connections can be found on the rear panel of the instrument.



Name	Function
Ethernet	Socket for connecting to the PC/laptop via an Ethernet cable.
On/Off Button	Turning the switch to position “I” switches on the instrument.
DC Input	Connector to the external power supply.

Preface

This manual is a guide for using Prometheus Series instruments and performing nanoDSF measurements. It instructs first-time users on how to use the instrument, and serves as a reference for experienced users.

Before using the Prometheus Series instrument, please read this instruction manual carefully, and make sure that the contents are fully understood. This manual should be easily accessible to the operator at all times during instrument operation. When not using the instrument, keep this manual in a safe place. If this manual is lost, order a replacement from NanoTemper Technologies GmbH.

Notices

1. NanoTemper Technologies shall not be held liable, either directly or indirectly, for any consequential damage incurred as a result of product use.
2. Prohibitions on the use of NanoTemper Technologies software:
    - Copying software for other than backup
    - Transfer or licensing of the right to use software to a third party
    - Disclosure of confidential information regarding software
    - Modification of software
    - Use of software on multiple workstations, network terminals, or by other methods
3. The contents of this manual are subject to change without notice for product improvement.
4. This manual is considered complete and accurate at publication.
5. This manual does not guarantee the validity of any patent rights or other rights.
6. If a NanoTemper Technologies software program has failed causing an error or improper operation, this may be caused by a conflict from another program operating on the laptop (PC). In this case, take corrective action by uninstalling the conflicting product(s).
7. NanoTemper is a registered trademark of NanoTemper Technologies GmbH in Germany and other countries.

Limited Warranty

Products sold by NanoTemper Technologies, unless otherwise specified, are warrantied to be free of defects in materials and workmanship for a period of one year from the date of shipment. If any defects in the product are found during this warranty period, NanoTemper Technologies will repair or replace the defective part(s) or product free of charge.

THIS WARRANTY DOES NOT APPLY TO DEFECTS RESULTING FROM THE FOLLOWING:

1. IMPROPER OR INADEQUATE INSTALLATION.
2. IMPROPER OR INADEQUATE OPERATION, MAINTENANCE, ADJUSTMENT OR CALIBRATION.
3. UNAUTHORIZED MODIFICATION OR MISUSE.
4. USE OF UNAUTHORIZED CAPILLARIES AND CAPILLARY TRAYS.
5. USE OF CONSUMABLES, DISPOSABLES AND PARTS NOT SUPPLIED BY AN AUTHORIZED NANOTEMPER DISTRIBUTOR.
6. CORROSION DUE TO THE USE OF IMPROPER SOLVENTS, SAMPLES, OR DUE TO SURROUNDING GASES.
7. ACCIDENTS BEYOND NANOTEMPER'S CONTROL, INCLUDING NATURAL DISASTERS.

This warranty does not cover consumables like capillaries, reagents, labeling kits and the like.

The warranty for all parts supplied and repairs provided under this warranty expires on the warranty expiration date of the original product. For inquiries concerning repair service, contact NanoTemper Technologies after confirming the model name and serial number of your NanoTemper Technologies instrument.

1. Prometheus Series Instruments

The Prometheus Series offers nanoDSF technology (see section 2). nanoDSF is the method of choice for easy, rapid and accurate analysis of protein stability and aggregation, with applications in protein engineering, membrane protein research, formulation development and quality control.

The Prometheus Series comprises two instruments. The Prometheus NT.48 is filled with up to 48 single capillaries for most flexible assay design and easy handling. The Prometheus NT.Plex employs 24-Capillary chips, which enables manual or automated sample loading. It can be coupled with robotic and liquid handling platforms for unattended operation and complete automation.

1.1. Excitation and Detection for Fluorescence Measurements

The Prometheus Series instruments use advanced UV-LEDs for the excitation of fluorophores, which are superior to UV-lasers in terms of stability, robustness and flexibility. Tryptophan fluorescence is collected by a proprietary Dual-UV detection unit which is optimized for the rapid and sensitive acquisition of tryptophan fluorescence at 330 nm and 350 nm.

LED	Fluorophore	Detection
UV (maximum at 280 nm)	Tryptophan, Tyrosine	Dual-UV detector: 330 nm and 350 nm
		Detection range: 0-20,000 fluorescence units

The detection unit scans the sample tray every 3 seconds, recording the fluorescence intensity at 330 nm and 350 nm (**Figure 1**). During a thermal unfolding experiment, the samples are measured continuously, yielding ultra-high resolution with more than 20 data points per minute. In case the instrument is equipped with the optional Aggregation Optics, scattering information is collected simultaneously and with equal data point density.

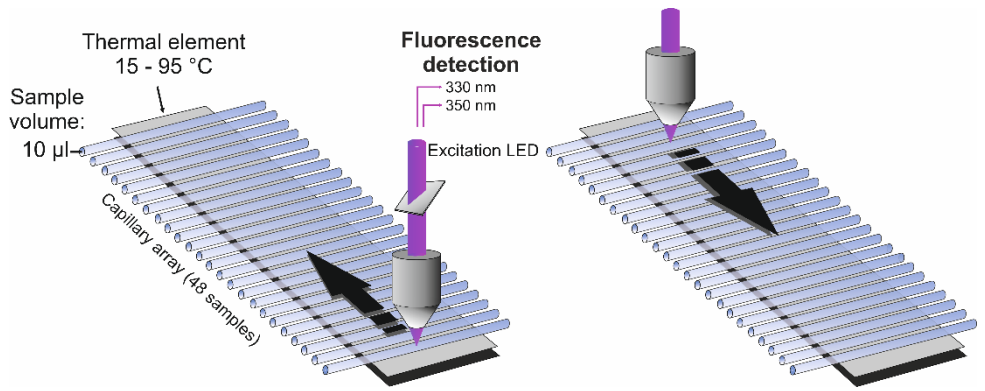


Figure 1: Prometheus high precision capillary format. Capillaries containing 10 µl sample are loaded onto a thermal element, which can be set to temperatures from 15 °C-95 °C. Within 3 seconds, the whole capillary tray is read by the Dual-UV detection unit.

1.2. Application Range

The Prometheus instruments use nanoDSF technology to detect changes in the fluorescence of the amino acids tryptophan and tyrosine over a wide range of temperatures (see section 1.4). The instruments are used to induce thermal unfolding of proteins and to determine thermal unfolding transition temperatures. Furthermore, thermal protein refolding can be monitored and refolding transition temperatures can be determined. The instruments can also be used for chemical unfolding and refolding experiments.

Optionally, the Prometheus instruments can be equipped with backreflection optics to investigate aggregation in a sample upon heating and to determine aggregation onset temperatures.

1.3. Sensitivity

The Prometheus instruments can measure a broad range of concentrations due to an adjustable LED excitation intensity.

Maximal protein concentration (standard IgG): > 250 mg/ml.

Minimal protein concentration (standard IgG): 5 µg/ml.

1.4. Temperature Range

The thermal element inside Prometheus instruments can be precisely adjusted from 15 °C to 95 °C. Additionally, the instruments can be equipped with a High Temperature Upgrade allowing a maximum temperature of 110 °C.

Note: *If experiments exceed a temperature of 95 °C, or measurement times of 3 hours, capillaries must be sealed using NanoTemper Technologies Capillary Sealing Paste (PR-P001) and Capillary Sealing Applicators (PR-P002).*

Note: *For instruments equipped with a High Temperature Upgrade: When running experiments with a maximum temperature of 110 °C, it is recommended to use heating rates of 1 °C/min or slower to ensure linearity of the temperature ramp. For all heating rates, ramp linearity is guaranteed up to at least 95 °C.*

Note: *For instruments equipped with a High Temperature Upgrade: When running experiments with a maximum temperature of 110 °C, the minimum ambient temperature is 20 °C to ensure the instrument reaches is target temperature.*

1.5. Precision

Temperature variance across capillary tray in thermal ramp of 1 °C/min: ± 0.2 °C (at 52 °C)

Run-by-run reproducibility of experiments in thermal ramp of 1 °C/min: ± 0.2 °C (at 52 °C)

1.6. Sample Consumption

The Prometheus instruments are designed to minimize the amount of sample needed. Only 10 µl of sample per capillary are required.

1.7. Capillary Format

The capillary format of the Prometheus instruments is cost-effective, easy to handle and offers maximal flexibility in the experiment scale, while offering a detection precision that is superior to other approaches. The number of samples measured in one run can be any number from one to 48 capillaries in a Prometheus NT.48 instrument, or one to 24 capillaries in a Prometheus NT.Plex instrument.

1.8. Dedicated Control and Analysis Software

The Prometheus instruments are supported by several software packages to enable straight-forward measurement setup and data analysis: the PR.ThermControl for thermal unfolding assays, the PR.ChemControl for chemical unfolding experiments, and the PR.TimeControl for (iso-)thermal stability analysis and experiments with non-linear temperature ramps or cycles. Please refer to the individual software manuals for detailed information on the respective kind of experiment.

2. nanoDSF Technology

nanoDSF is an advanced Differential Scanning Fluorimetry technology. It detects smallest changes in the fluorescence of tryptophan and tyrosine residues present in virtually all proteins. The fluorescence of tryptophans and tyrosines in a protein is strongly dependent on their close surroundings. When located in the hydrophobic core of proteins, tryptophan is shielded from the surrounding aqueous solvent. Upon unfolding however, tryptophan is exposed, which alters its photo-physical properties. By following changes in fluorescence, the unfolding of proteins can be monitored in real time in a truly label-free fashion (**Figure 2**).

Thermal and chemical unfolding experiments are highly appreciated methods to quantify protein stability. While thermal unfolding experiments use a temperature ramp to monitor protein conformational changes, chemical unfolding experiments use chaotropes such as urea to unfold proteins. Both types of experiments are easily done with the Prometheus Series instruments.

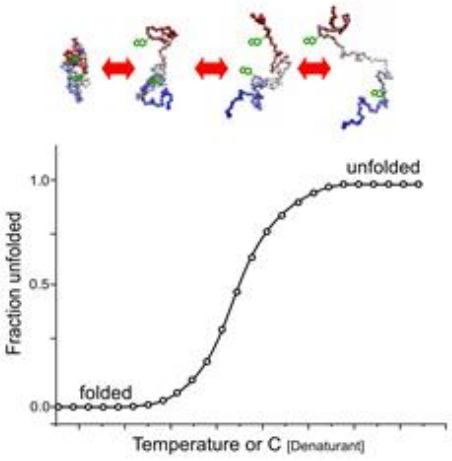


Figure 2: Schematic representation of protein unfolding. Charged, solvent exposed regions of the protein are colored in red and blue, the hydrophobic core in gray. Tryptophan residues are represented as green sticks. The illustration shows their increasing exposure to the solution upon unfolding. Below, the respective curve shows the transition from folded to unfolded protein as a function of temperature or the concentration of a chemical denaturant.

The unique dual-UV technology of the Prometheus Series monitors intrinsic tryptophan and tyrosine fluorescence at the emission wavelengths of 330 nm and 350 nm. To generate an unfolding curve, either the fluorescence change in one of the two channels, or, alternatively, the ratio of the fluorescence intensities (F350 nm/F330 nm), is plotted against temperature or concentration of denaturant (see **Figure 2**). The fluorescence ratio monitors changes in fluorescence intensity as well as the tryptophan-specific shift of the fluorescence emission maximum. It is therefore extremely robust and allows the investigation of virtually all buffer conditions and even fluorescent additives.

3. Using the Prometheus Series Instruments

3.1. General Usage

Start the Prometheus instrument using the power switch at the back left of the instrument, turn on the control computer and start the software (PR.ThermControl, PR.TimeControl or PR.ChemControl). It will take approximately one minute for the Prometheus instrument to complete hardware initialization. You can use the touch display of the Prometheus instrument to set the internal temperature to a desired value, and to open the capillary tray drawer. These commands can also be performed in the control software.

After the experimental session is finished, turn the Prometheus instrument off using the switch. Shut down the control computer. No particular sequence needs to be followed.

Note: *No equilibration times are required after startup. Prior to temperature ramps, the Prometheus instruments automatically perform a one minute equilibration routine.*

Note*: Always remove capillaries and capillary lids prior to transport of the instrument.*

3.2. Sample Loading

Clean the mirrored surface of the capillary tray with a scratch- and dust-free tissue and 99.8 % ethanol prior to experiments. Keep the tray surface free from dust, dirt and scratches. In order to detect protein unfolding, proteins must contain at least one tryptophan or other fluorescent residues that show changes in their fluorescent properties in the dedicated detection range. 10 µl of sample are required per capillary for unfolding experiments. Capillaries (or capillary chips) are dipped into the sample and automatically fill by capillary forces. Capillary filling is accelerated by holding the capillary horizontally. Make sure that capillaries are filled completely. Tolerable capillary filling levels for thermal unfolding experiments are shown in **Figure 3** (top right panel). Once capillaries are filled, place them onto the capillary tray and fix the capillaries by placing the lid onto the tray. Differences in sample loading between different Prometheus Series instruments are described in the following sections.

Note: *NanoTemper Technologies offers two different types of capillaries, nanoDSF Grade Standard Capillaries and nanoDSF Grade High Sensitivity Capillaries. To improve sensitivity at sample concentrations < 200 µg/ml, the nanoDSF Grade High Sensitivity capillaries are recommended.*

Note: *Avoid liquid on the outside of the capillary, since this will alter the fluorescence signal. If there is liquid on the outside of the capillaries, use a dust- and scratch-free tissue to wipe the capillaries clean.*

Note*: In case of broken capillaries on the capillary tray, use a paintbrush or tissue to carefully remove pieces of glass and to avoid scratching the tray.*

3.2.1. Sample Loading for the Prometheus NT.48

The Prometheus NT.48 capillary tray provides 48 numbered slots for single capillaries, offering maximum flexibility in assay design. It is important that the capillaries are positioned in the center of the tray. It is also important that the meniscus of the sample does not approach the heated capillary tray surface, since this will lead to rapid evaporation (**Figure 3**).

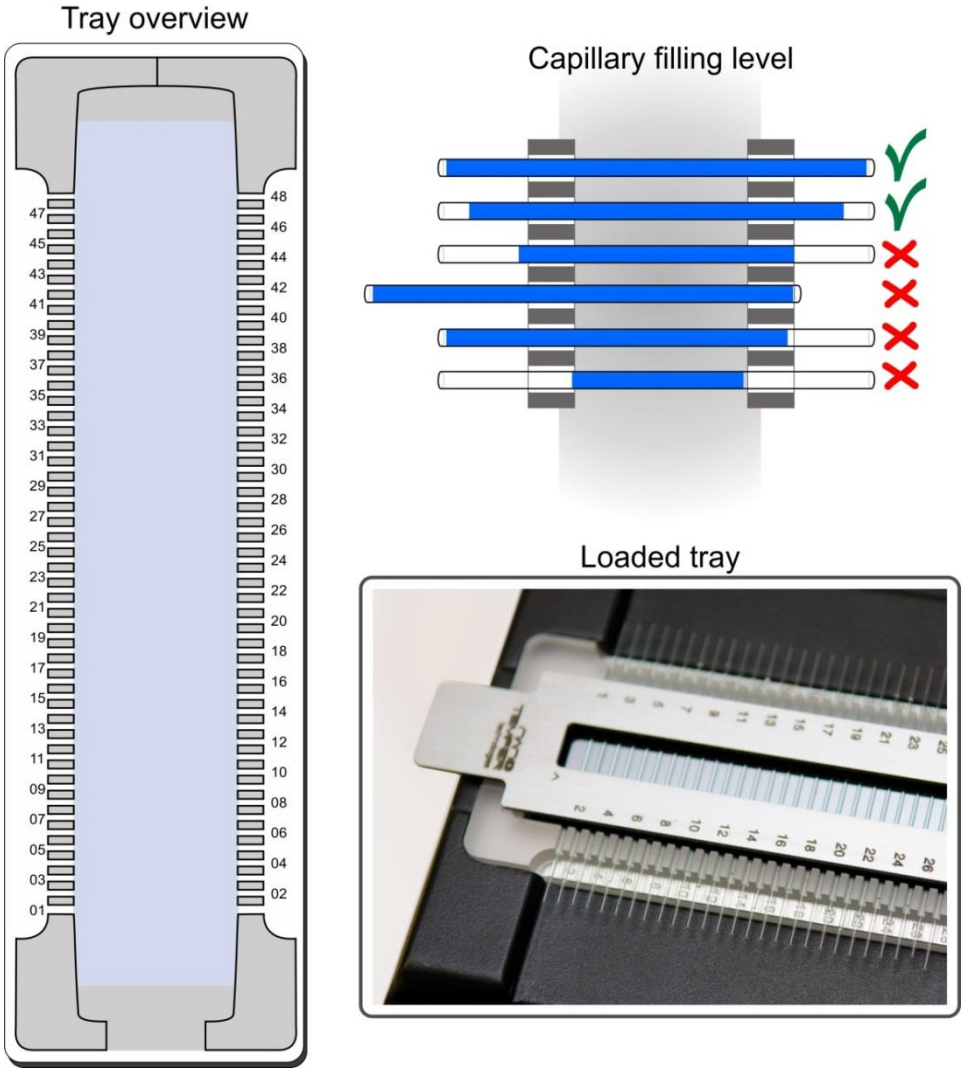


Figure 3: Capillary tray layout and loading for the Prometheus NT.48 instrument. The capillary tray can hold up to 48 capillaries, which are numbered from 1 to 48, starting in the front closest to the user. When filling the capillaries, it is important that the capillaries are filled completely, and that they are centered on the capillary tray. Once all capillaries are loaded onto the capillary tray, place the magnetic lid to fix the capillaries.

3.2.2. Manual Sample Loading for the Prometheus NT.Plex

The Prometheus NT.Plex instrument is designed for fast, automatable high-throughput measurements. Sample loading can be performed automatically (see 3.2.3) or manually, as described in this section. The capillaries on Prometheus NT.Plex nanoDSF Grade 24-Capillary Chips are spaced to enable easy and fast sample loading from 384-well microtiter plates. The Capillary Chip Filling Station (Cat# NT-AT100) is recommended for manual loading of capillary chips from microtiter plates since it places the sample at the optimal angle for loading (see

Figure 4 B). Correct positioning of the capillaries on the tray is ensured by the capillary chip format.

Place the chip onto the thermal element with the capillaries facing upward (see **Figure 4** A). Use the number ① imprinted on the chip for orientation, for example to load your sample number 1. Use the symbols next to the thermal element for correct chip placement. It is recommended to place the capillary chip with the imprinted number ① toward the user, since the capillary closest to the user will be designated “capillary 1” by the software.

The arrow imprinted on the chip indicates the capillary type. An arrow pointing upward (toward the ①) designates standard capillaries while an arrow pointing left (away from the capillaries) designates high sensitivity capillaries. See 3.2 for recommendations on which capillaries to use.

Next, place the lid slowly and evenly inside of the guiding structures (see **Figure 4** A). The weight of the lid holds the capillaries in place. Slight movements of the lid (and the capillary chip) inside of the guiding structures are normal and will not affect the measurement.

CAUTION: Careless, hasty or diagonal placement of the lid can lead to damage of capillaries or the thermal element. Keep hands away from the instrument when closing the sample drawer. Especially, be careful not to get caught between capillary lid and instrument, since this can lead to injury and instrument damage. If necessary, push the ‘Cancel’ button on the touch display of the instrument to stop the drawer from closing.

Figure 4: Manual sample loading for the Prometheus NT.Plex. (A) When placed correctly, the plastic chip structure frames the thermal element and the capillaries rest on top of the thermal element. The metal lid is then placed slowly and evenly inside of the guiding structures, with the rubber padding securing the capillaries in place. The red arrow indicates the imprinted number ① on the capillary chip. (B) The Capillary Chip Filling Station is recommended for manual loading of capillary chips from microtiter plates.

3.2.3. Automated Sample Loading for the Prometheus NT.Plex

The Prometheus NT.Plex instrument can be coupled with the NT.Robotic Autosampler for automated sample loading. For details, please see the NT.Robotic Autosampler manual.

4. Patents and intellectual property

Prometheus and the nanoDSF technology are patent protected, especially by the following patents, US10488326B2, US10545081B2, US10618051B2, US10900879B2, EP2848310B1, EP3150988B1, including their application and registration in different other countries.

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