

# What is included in this product manual

# **PROMETHEUS MANUALS**

User Manual

# **PROMETHEUS CAPILLARIES**

Accessory Manual

# TUTORIALS

FAQs



# **PROMETHEUS PANTA**

User Manual

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# 1. About this user manual

This manual is a guide for using Prometheus Panta instrument. It instructs firsttime users on how to use the instrument and serves as a reference for experienced users.

Before using the Prometheus Panta instrument, please read this instruction manual carefully, and make sure that the contents are fully understood. This manual should <u>be easily accessible to the operator at all times during instrument</u> <u>operation</u>. When not using the instrument, keep this manual in a safe place. In case of loss, please visit the NanoTemper Technologies **Support Center** (https://support.nanotempertech.com/hc/en-us) for a replacement copy of this manual.

# 1.1. Directions for more detailed information

There are two sources for more detailed information on scientific principles and recommendations for software usage, as well as data interpretation. One is the **NanoPedia** knowledge base that is integrated into the PR.Panta Control software. It contains a variety of articles about the biochemistry of protein stability, the implemented measurement technologies nanoDSF, backreflection, DLS and SLS and the operational functions of Prometheus Panta.

For further, more detailed information on scientific principles and recommendations for assay development as well as software usage visit our **Support Center (https://support.nanotempertech.com/hc/en-us)**.

The NanoTemper Technologies **Support Center** is an online community to obtain resources for NanoTemper products such as application-notes, tech-notes or protocols. You can also pose questions and have them answered by NanoTemper support guides.



# 2. Safety information

To ensure operation safety, this system must be operated correctly. Carefully read this chapter to fully understand all necessary safety precautions before operating the system.

#### 2.1. Symbols and descriptions

This section describes the safety symbols and descriptions used in this manual, as well as the labels on the system.

Please take a moment to understand what the signal words **WARNING! CAUTION** and **NOTE** mean in this manual.

- **WARNING!** A **WARNING!** indicates a potentially hazardous situation which, if not avoided, could 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 system.

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

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



Read manual label. This label indicates that you must read the manual before using the system. This label is positioned on the back of the instrument.



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.

#### 2.2. Use and misuse

Use the Prometheus Panta system only after having read and fully understood this user manual. Use the system only in perfect condition. If the system shows any signs of damage, stop operation and contact NanoTemper Technologies GmbH.

Do not modify the system in any way. Do not use it for anything other than its intended purpose.

#### 2.3. Safety instructions

**CAUTION** The Prometheus Panta <u>must</u> be installed in a way that does not hinder access to the external power supply and its power plug.

**CAUTION** Connect the Prometheus Panta 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.

**CAUTION** Do not operate the Prometheus Panta instrument with a power supply other than the one delivered by NanoTemper Technologies.

**WARNING!** Connect the Prometheus Panta to the AC power supply using the supplied power cable.

The AC power supply, which is connected to the Prometheus Panta, must only be connected to an outlet that has a ground contact, since the instrument is assembled in line with the specifications for safety class IEC 61010-1:2010.

**WARNING!** Danger of electric shock, fire and skin burns. Do not open the system (other than operating the door via the software). Do not reach into the door opening.

**WARNING!** Do not operate the Prometheus Panta 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 Panta instrument weighs approx. 35 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. Do not reach into the opening when the door is open.

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

**CAUTION** The sharp edges of the Prometheus Panta 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 (20-70 % 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 100% ethanol or other types of organic solvents to clean the instrument as they may remove the instrument paint.

**CAUTION** The instrument contains a laser module (visible laser radiation class 3B according to IEC 60825-1: 2014). Lasers or laser systems emit intense, coherent electromagnetic radiation that has the potential of causing irreparable damage to human skin and eyes. Direct eye contact can cause corneal burns, retinal burns, or both, and possible blindness. Do not attempt to open the instrument as this poses a risk of personal injury or damage to the instrument. When the instrument is used as intended it emits laser radiation of LASER CLASS 1.



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

**CAUTION** The heating element contains nickel. If allergic to nickel wear protective gloves when cleaning the tray surface to avoid skin irritation and damage.

**CAUTION** If liquids penetrate the instrument, for example due to spillage, stop operation of the instrument, disconnect it from the power supply and contact NanoTemper Technologies GmbH.

**NOTE** Insufficient air supply can cause overheating of the system. Assure enough air supply by not covering or obstructing the back of the system.

**NOTE** Do not install the instrument in a location where it may be exposed to direct sunlight.

# 3. The Prometheus Panta system

#### 3.1. General

#### 3.1.1. Intended use

The Prometheus Panta system provides fast and sensitive determination and quantification of conformational and colloidal protein stability. For this purpose, it performs nanoDSF (nano-differential scanning fluorimetry), backreflection, DLS (dynamic light scattering), and SLS (static light scattering) measurements in glass capillaries. The system is intended for research purposes only. It is not to be used for diagnostic purposes.

#### 3.1.2. Conformity

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 61326-1:2012 EMC, Electrical equipment for measurement, control and laboratory use EMC requirements.
- · IEC 60825-1:2014 Safety of laser products.
- Complies with 21 CFR 1040.10 and 1040.11 except for conformance with IEC 60825-1 Ed. 3., as described in Laser Notice No. 56, dated May 8, 2019

#### 3.1.3. Identification

The identification labels (Figure 1) are positioned at the rear panel of the instrument. They include manufacturer information, system model name and serial number (SN), electrical requirements, and the CE and NRTL conformity symbol.





Figure 1: Identification labels for Prometheus Panta.

#### 

# 3.2. Technical information

# 3.2.1. Technical specifications

Electricity		Power supply dimensions	
Input of External Power Supply	90–264 VAC ± 10 % 47–63 Hz,	Width	21 cm (8.3")
	230 VA max	Height	9 cm (3.5")
Output of external power supply	24 VDC, 11.67 A max	Depth	3 cm (1.1")
Electrical input to Andromeda	24 VDC, 10 A	Weight	0.5 kg (1.1 lbs) net max
Series instrument		DLS Laser	
Pollution degree	2	Wavelenght	405 nm ± 5 nm
Environmental		Power	100 mW max
Operating temperature	15–30 °C (indoor use only)	Dromothous Donto	
Humidity	20-70%, noncondensing	Laser classification	CLASS 1
Operating altitude	max 2000 m	Temperature control	
Prometheus Panta dimensions		Temperature control range	15 °C – 110 °C (at 25 °C)
Width	35 cm (13.8")		
Height	51 cm (20.8")	Precision of temperature control	± 0.1 °C
Depth	55 cm (20.5")		
Weight	35 kg (77 lbs) net		

#### 3.2.2. Connections for input and output

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

Туре	Function
Ethernet	To connect the system to the Control PC/LAN via Ethernet cable.
On/Off Button	To connect the system to electrical power.
DC Input	Turning the switch to position "I" switches on the instrument.

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



Figure 2: Connections on the Prometheus Panta device.

#### MOTEMPER

#### 3.3. Legal

- 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 purposes 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 content of this manual is 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 controlling PC. In this case, take corrective action by uninstalling the conflicting product(s).
- 7. NanoTemper and Prometheus are registered trademarks of NanoTemper Technologies GmbH.
- 8. Unauthorized resale is not permitted.

#### 3.4. 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 identified 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 microwell plates and accessories.
- 5. Use of consumables, disposables and parts not supplied by an authorized NanoTemper Technologies distributor.
- 6. Corrosion due to the use of improper solvents, samples, or due to surrounding gases.
- 7. Accidents beyond NanoTemper Technologies' control, including natural disasters.

This warranty does not cover consumables like microwell plates, reagents, labeling kits and the like. It also does not cover normal wear-and-tear.

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 system (see 3.1.3).

# 4. Prometheus Panta setup

The Prometheus Panta should be installed by NanoTemper Technologies personnel to ensure safety measures are taken and to confirm proper functionality of the instrument.

# 4.1. Scope of delivery

Upon receiving the system, please check package contents for completeness. The Prometheus Panta system package contains the following items:

ltem	Description
Prometheus Panta system	-
User manual	This user manual
Power Supply and cables	24V power supply, network cable for connection to control PC
Control PC	Laptop for Prometheus Panta system

#### 4.2. Unpacking

The Prometheus Panta system should only be unpacked and installed by trained NanoTemper Technologies personnel to ensure proper functionality of the instrument upon delivery.

#### 4.3. Startup

Connect the Prometheus Panta system to power by plugging in the 24V power supply. Connect the Prometheus Panta system to the control PC using the Ethernet connection at the back of the instrument. The system starts upon switching the power switch.

# 4.4. Cleaning

The Prometheus Panta system does not need any regular maintenance.

To clean the outside surface of the system, unplug the power supply at the back. Wipe the surface, including the front display, with a cloth slightly dampened with water or ethanol (70%).

The capillary tray, including its mirrored surface, can be cleaned when the device is switched on and the capillary tray drawer is in the open position (see 6.1). Clean the mirrored surface of the capillary tray with a scratch- and dust-free tissue and 99.8 % ethanol prior to experiments.

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. Other surfaces of the capillary tray can be cleaned in a similar fashion as the outside of the device.

# 4.5. Software updates

Software updates of the embedded system can only be performed by instructed NanoTemper Technologies personnel and is part of regular maintenance visits. Software updates of PR.Panta Control and PR.Panta Analysis software can be performed by the users within the respective software solution.

# 5. Prometheus Panta instrument

The Prometheus Panta instrument uses nanoDSF technology to detect changes in the fluorescence of the amino acids tryptophan and tyrosine over a wide range of temperatures. The instrument is 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.

Secondly, the Prometheus Panta instrument is equipped with backreflection optics to investigate aggregation and precipitation in a sample upon heating and to determine aggregation onset temperatures.

Thirdly, the Prometheus Panta instrument is equipped with DLS (dynamic light scattering) and SLS (static light scattering) optics to investigate the particle size and particle size distributions at constant temperature. Furthermore, particle sizes and size distributions can be measured during thermal unfolding in parallel to nanoDSF and backreflection to determine onset temperatures of size changes.

#### 5.1. Temperature Range

The thermal element inside Prometheus instruments can be precisely adjusted from 15  $^{\circ}\mathrm{C}$  to 110  $^{\circ}\mathrm{C}.$ 

**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:** When running experiments with a maximum temperature of 110 °C, the minimum ambient temperature is 20 °C to ensure the instrument reaches its target temperature.

**Note:** For measurement temperatures below 20°C, the ambient temperature should be  $\leq 25^{\circ}$ C to ensure the instrument reaches target temperature.

## 5.2. Capillary Format

The capillary format of the Prometheus Panta 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 or one to 24 capillaries when using capillary chips. Prometheus systems should only be used in conjunction with high quality glass capillaries or capillary chips provided by NanoTemper Technologies.



## 6. Using Prometheus Panta

#### 6.1. General Usage

Start the Prometheus Panta instrument using the power switch at the back left of the instrument, turn on the control computer and start the software (PR.Panta Control). It will take approximately one minute for the Prometheus instrument to complete hardware initialization. You can use the touch display of the 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 Panta 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.

#### 6.2. 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. 10  $\mu$ l of sample are required per capillary for an experiment. Capillaries 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 4 (top right panel). Once capillaries are filled, place them onto the capillary tray and fix the capillaries by placing the lid onto the tray.

**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.

#### 6.2.1. Sample Loading for the Prometheus Panta

The Prometheus Panta offers maximum flexibility in assay design since it is compatible with single capillaries as well as capillary chips. The removable capillary frame provides 48 numbered slots for single capillaries. 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).

# CAPILLARY FRAME





**Figure 3:** Capillary tray layout and loading for the Prometheus Panta instrument. The removable capillary frame 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.

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Alternatively, samples can be loaded into capillary chips. The capillaries on Prometheus 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.

Next, place the lid slowly and evenly on top of the capillary chip (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 of capillary chips. (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 on top of the capillary chip. 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.

# 7. Patents and intellectual property

Prometheus and the nanoDSF technology are patent protected, especially by the following patents, US10618051, US10488326B2 including their application and registration in different other countries.

# 8. Transport and disposal

#### 8.1. Repackaging for transport

The Prometheus Panta instrument should be repacked only by trained NanoTemper Technologies personnel to ensure safety and stability during transport. Please store the instrument box for that purpose. If the instrument box was discarded NanoTemper Technologies can provide replacement at the cost of shipment.

### 8.2. Waste disposal

Please dispose of used glass capillaries according to the substances contained in them and according to locally applicable regulations concerning chemical and glass waste.

### 8.3. System disposal

The system may need to be decontaminated before disposal. Please contact NanoTemper Technologies for more information.



This symbol indicates that this system may not be disposed of as unsorted municipal waste and must be collected separately. It must be disposed of according to locally applicable regulations regarding electrical and electronic equipment. The symbol is positioned at the back of the instrument.

#### 

# Contact

NanoTemper Technologies GmbH

Global headquarters: Toelzer Strasse 1, 81379 Muenchen | Germany

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

info@nanotempertech.com nanotempertech.com

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# **PROMETHEUS CAPILLARIES**

Accessory Manual

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# 1. Prometheus<sup>®</sup> Capillary Chips Kits

#### 1.1. General Information

NanoTemper Technologies offers special Capillary Chips with high quality and verified functionality. Our Capillary Chips are developed for usage in Prometheus NT.Plex and Prometheus Panta. It is highly recommended to use original Prometheus Capillary Chips for qualitative and reproducible measurements. The Prometheus warranty is only valid if the instrument is used with original Prometheus Capillary Chips.

#### 1.2. Content

• 8 Capillary Chips (24 capillaries each)

#### 1.3. Handling and Storage

- · Store at room temperature
- · Store in a dark and dry place
- Do not touch the Capillary Chips in the capillary center. Hold them from the ends
- Use gloves
- · Capillary Chips are for single use only
- · Used Capillary Chips must be disposed of in accordance with federal, state and local environmental control regulations
- · Important: Always close Capillary Chip box to keep Capillary Chips dust-free

#### **1.4. Security Advice**



Be careful when using Capillary Chips.

Broken capillaries are broken glass and can be harmful to skin and eyes.

### 1.5. Capillary Type

Cat #	Capillary Type
PR-AC002	Standard
PR-AC006	High Sensitivity

#### 1.6. Help

For further information, please refer to our Prometheus User Manual or contact our NanoTemper application support:

**Call:** +49-(0)89-4522895-0

#### Email: info@nanotempertech.com

# 2. Prometheus<sup>®</sup> Capillary Kits

#### 2.1. General Information

NanoTemper Technologies offers special Capillaries with high quality and verified functionality. Our Capillaries are developed for usage in Prometheus NT.48 and Prometheus Panta. It is highly recommended to use original Prometheus Capillaries for qualitative and reproducible measurements. The Prometheus warranty is only valid if the instrument is used with Prometheus Capillaries.

#### 2.2. Content

- · 1x (PR-C006) vial of Capillaries (200 each)
- or
- · 2x (PR-C002) vials of Capillaries (200 each, 400 total)

#### 2.3. Handling and Storage

- · Store at room temperature
- · Store in a dark and dry place
- · Do not touch the center of the capillaries. Hold them from the ends
- Use gloves
- · Capillaries are for single use only

- · Used capillaries must be disposed of in accordance with federal, state and local environmental control regulations
- · Important: Always close capillary vials to keep capillaries clean and dust-free

#### 2.4. Security Advice



Be careful when using capillaries

Broken capillaries are broken glass and can be harmful to skin and eyes.

# 2.5. Capillary Type

Cat #	Capillary Type
PR-C002	Standard
PR-C006	High Sensitivity
2.6. Help	
For further information, please refer to our our NanoTemper application support:	<sup>·</sup> Prometheus User Manual or contact
<b>Call:</b> +49-(0)89-4522895-0	
Email: info@nanotempertech.com	

CAT NR: PR-P001

# 3. Prometheus<sup>®</sup> Series Capillary Sealing Paste

nanoDSF<sup>®</sup> Grade

Kit for the sealing of capillaries for high temperature or long-term experiments for use in nanoDSF  $^{\mbox{\scriptsize \$}}$ 

Content andPrometheus Series Capillary Sealing Paste is shipped at<br/>room temperature. Each kit contains material sufficient for<br/>approximately 50 sealing procedures.

5 x 15 g Capillary Sealing Paste

Expiry date: see kit cover.

Capillary sealing paste can be used for at least three months after opening the tube.

SafetyPrometheus Series Capillary Sealing Paste is an oil-basedIntructionsliquid rubber. Keep away from skin and eyes. Do not eat. Easily<br/>flammable. Wear protective gloves when handling the sealing<br/>paste.

#### 3.1. Important Information Before Starting

This kit is designed to seal capillaries for experiments using Prometheus instruments. For standard thermal unfolding experiments (measurement times < 3 h, temperatures  $\leq$  95 °C), sealing of capillaries is not required. Long term measurements > 3 h or thermal unfolding experiments which exceed a temperature of 95 °C require sealing of capillaries to prevent air-bubble formation and/or extensive evaporation.

Prometheus Capillary Sealing Applicators (PR-P002, sold separately) are required for capillary sealing.

The sealing paste contained in this kit will not influence your measurements in any way since it is chemically inert, silicone-free, and water-insoluble.

#### 

# 3.2. Safety Information

# **Capillary Sealing Paste**

	Hazard statements		P264	Wash hands thoroughly after handling.
	H225	Highly flammable liquid and vapour.	P271	If eye irritation persists: Get medical advice/attention.
	H304	May be fatal if swallowed and enters airways.	P273+P501	Avoid release to the environment.
	H315	Causes skin irritation.		Dispose of content/container to an appropriate recycling and disposal
	H319	Causes serious eye irritation.		facility.
$\wedge$	H336	May cause drowsiness or dizziness.	P280	Wear protective gloves/ protective clothing/eye protection/face protection.
$\checkmark$	H361f	Suspected of damaging fertility.	P301+P310	IF SWALLOWED: Immediately call a
•	H373	May cause damage to organs through		POISON CENTER/ doctor.
		prolonged or repeated exposure.	P331	Do NOT induce vomiting.
	H412	Harmful to aquatic life with long lasting		
		effects.	For more information, please consult	the respective Safety Data Sheets (SDS).
	Precautionary state	ments	SDS are available from NanoTemper Te	chnologies upon request.
	P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.		
	P235	Keep cool.		

P260Do not breathe dust/ fume/ gas/ mist/<br/>vapours/ spray.

### 3.3. Capillary Sealing Procedure

- 1. Fill capillaries with sample and place them on the capillary tray. Make sure capillaries are aligned and fix them with the magnetic lid (see the Prometheus Series Manual for more information on sample loading).
- 2. Always wear protective gloves when handling the sealing paste. Apply sealing paste to the cavity of both sealing paste applicators.



3. (A) Place the applicators next to the capillary tray with the NanoTemper Technologies logos facing upward and the sealing paste toward the capillaries.(B) Slide the applicators toward the tray until all capillaries dip into the sealing paste. (C) Withdraw applicators.



- 4. Let the first layer dry for five minutes, then repeat. Always apply two layers of seal. One loading of paste to the applicators is enough for the complete sealing process, as the sealing paste on the applicators will not dry within the application time.
- 5. Let seal dry for another five minutes and remove applicators from the Prometheus Series drawer before starting the experiment.
- 6. Run the unfolding experiment.
- 7. After the sealing paste has dried (1-2 h), remove it from the applicators and discard. The applicators can be reused.

#### NOTE

When the capillary sealing paste tube is opened after prolonged storage, the first few drops to emerge may be a colorless liquid. Discard this liquid and use only the white sealing paste for capillary sealing.

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#### 3.4. Contact

#### 3.4.1. TECHNICAL SUPPORT

Please get in touch with us for specific questions concerning the product performance.

NanoTemper Technologies GmbH Toelzer Strasse 1, 81379 Muenchen | Germany

**Phone:** +49 (0)89 4522895 0 **info@nanotempertech.com** 

#### 3.4.2. PURCHASER NOTIFICATION

NanoTemper Technologies grants the buyer the non-transferable right to use the purchased product for research conducted by the buyer. The buyer cannot sell or otherwise transfer this product or its components for commercial purposes.

#### Limited warranty:

NanoTemper Technologies will replace any product that does not meet the specifications. This warranty limits NanoTemper Technologies' liability only to the cost of the product when within the expiration date.

No warranty is applicable unless all product components are stored in accordance with instructions.

NanoTemper Technologies assumes no responsibility or liability for any indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive.

#### For information contact NanoTemper Technologies GmbH

Toelzer Strasse 1, 81379 Muenchen | Germany

Phone: +49 (0)89 4522895 0 info@nanotempertech.com

For ordering NanoTemper products:

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

order@nanotempertech.com

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**TUTORIALS** 

FAQs

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# 1. How to Prepare Samples for Prometheus Panta

#### 1.1. General sample handling

For every Prometheus Panta experiment, please carry out the following steps:

#### · Clean the capillary tray surface

99.8% ethanol Dust- and scratch-free tissues

- · Load capillaries completely and align them centered before placing the lid
- · Avoid liquid on the outside of the capillary

As always, avoid scratches to the surface of the tray.

### 1.2. Sample preparation

Prometheus Panta performs measurements over a large protein concentration range. We recommend using 1 mg/mL as a starting concentrations for all thermal denaturation experiments. For proteins with  $\leq$ 1 Tryptophan for nanoDSF; or for proteins <50 kDa in size for DLS/SLS, increase the starting concentration.

For DLS measurements it is important to avoid dirt and dust as they will directly interfere with the measurement and create artefacts in the data. For this reason, we recommend the following:

- · Keep capillary vial closed when not in use
- · Use a fresh box of pipet tips and a fresh bag of sample tubes or plates
- · Keep boxes for pipet tips or tube lids closed when not in use

· Filter all buffers (see below)

· Filter samples or spin down (see below)

#### 1.2.1. Filtration of water and buffers

To record high-quality DLS data, it is advisable to work with highly pure water (e.g. AnalaR NORMAPUR; VWR **#102927G**) and buffer components. Filter water and buffer prior to usage with filters having a cutoff of 0.2  $\mu$ m (e.g. Filter devices: Nalgene 25 mm; Thermo Fisher Scientific **#724-2020** or Nalgene 13 mm; Thermo Fisher Scientific **#720-1320**)

To get the best possible result, filter water or buffer twice through the same filter into fresh and clean 15 mL/ 50 mL tubes.

#### 1.2.2. Pre-rinsing sample tubes

Similar to filtering water and buffer, keeping sample solutions in clean and dustfree sample containers can improve DLS data quality. When unsure whether the sample or buffer containers are free of any particulate matter or when dustartifacts are detected in the data despite having filtered all solutions, try prerinsing sample containers:

- 1. Place sample tubes in sample holder
- 2. Set a pipet to the matching volume
- 3. Rinse the pipet tip once with filtered water, discard
- 4. Use a clean tip to fill filtered water into all tubes
- 5. Clean tubes by shaking / inverting

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- 6. Discard water by tipping it out (make sure no water remains in the tube, e.g. in the lid)
- 7. Close tubes again to avoid contamination

#### 1.2.3. Filtration of protein solutions

Working with dirt- and dust-free sample solutions is key to determine the correct size of particles in a sample. It is therefore advisable to filter protein solutions directly before starting an experiment.

An alternative method to remove dirt and dust is spinning down the sample directly before the experiment (e.g., 14.000g, 10-15 minutes at 4° C in a tabletop centrifuge).

When filtering protein solutions, we recommend carrying out the following steps:

- 1. Start with clean tubes (as described under 1.2.)
- 2. Filter the protein solution:
- For standard DLS application, filter protein solution through 0.22 μm pore size filters witha sufficiently small dead volume. e.g. Nalgene 13 mm; Thermo Fisher Scientific #720-1320 or Costar Spin-X Centrifuge Tube filter; Corning Inc. # CLS8161-100EA
- For optimal DLS results, filter protein solution through 0.02 μm pore size filter e.g. Whatman Anotop 10 Plus; GE Healthcare **#6809-3002**
- 3. Take a fresh, clean 1 mL syringe, fill the syringe with the protein solution and filter it into the clean tube (discard first droplet or subsequently filter the sample for a 2nd time)

#### Please note:

For all subsequent pipetting / dilution steps, always use clean tubes and pipet tips pre-rinsed with filtered water!

Whether or not to filter depends on the application. If, for example, one is interested in the long-term stability of proteins, one might want to filter the sample at the beginning of the experiment, but then keep it as it is for all subsequent measurements to assess if larger particles form over time.

# 2. How to transfer sofware licenses

Licenses can be moved to a different user account or to a different PC. To transfer a license to a different user account (or a different PC), first deactivate a copy of a license that is currently activated, before reactivating a license.

#### 2.1. Deactivation of license

To deactivate a license, open the software and click on 'License Status' in the top left corner.



In a new window named 'Licensing Status', click on 'Deactivate now'.



A deactivation code will be displayed. Please send this deactivation and the order conformation number or invoice number from the purchase of your license to **order@nanotempertech.com** 

### 2.2. Reactivating a license

To reactive a license, install a copy of a software on the new computer (this step is not needed if the license is transferred to a new user account on the same PC). Open the software and go to 'License Status' tab to access a new 'Request code'. Please send the **new request code** to **order@nanotempertech.com**, together with **order conformation number** or **invoice number** from the purchase of your license. You will receive an e-mail with an activation code for your request code. Enter the activation code in the 'Activation code' box and click 'Activate'.

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Request code: Activation code:	Please enter

**Tip!** Deactivation code and a new request code for reactivating a license can be sent at the same time in one email to **order@nanotempertech.com**.

#### 

# Contact

NanoTemper Technologies GmbH

Global headquarters: Toelzer Strasse 1, 81379 Muenchen | Germany

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

info@nanotempertech.com nanotempertech.com

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# NanoTemper Technologies GmbH

Global headquarters:

Toelzer Strasse 1, 81379 Muenchen | Germany

**Phone:** +49 (0)89 4522895 0

**Fax:** +49 (0)89 4522895 60

info@nanotempertech.com

nanotempertech.com

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