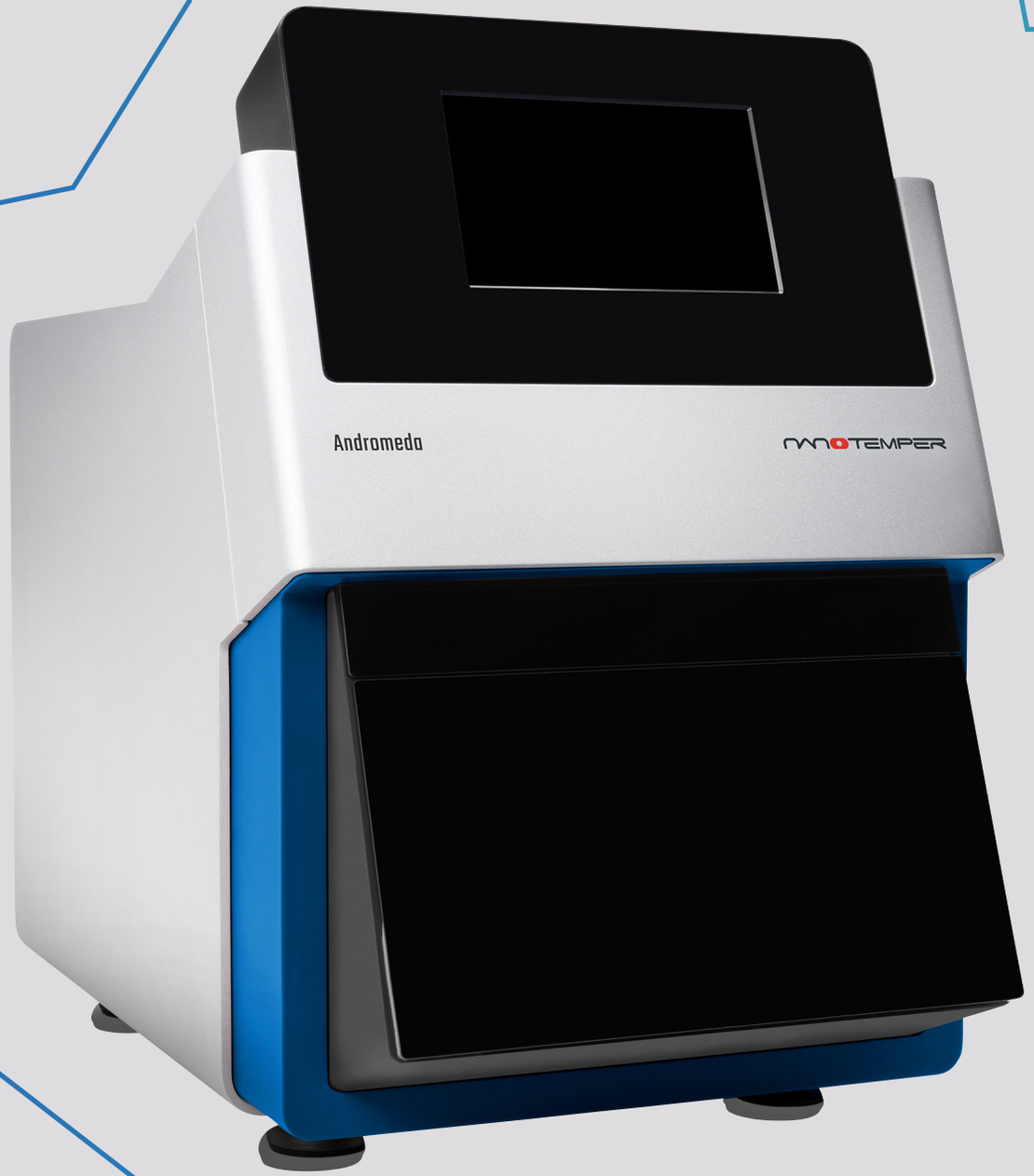

ANDROMEDA

PRODUCT MANUAL

What is included in this product manual

ANDROMEDA

User Manual

Table of contents

1. About this user manual

This user manual gives guidance on the correct use of the Andromeda and Andromeda Plex systems. It covers system specifications, safety considerations and installation as well as why and how to run experiments with Andromeda. Please read this manual carefully before starting and make sure the contents are fully understood. Keep this manual available near the system for future reference. In case of loss, please contact NanoTemper Technologies customer support (**nanotempertech.com/support**) for a replacement copy of this manual.

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, when used on its own or in conjunction with any of the following icons indicates the need to consult the provided manual, because a potential risk exists if the operating instructions are not followed. This label is positioned on the back of the instrument.

Warning symbol. This symbol indicates moving parts that can crush and cut. This warning label is positioned on the instrument tray holding the microwell plate.

2.2. Use and misuse

Use the Andromeda 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 customer support.

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

2.3. Safety instructions

WARNING! Operate the Andromeda 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 Andromeda 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 Andromeda 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 Andromeda 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 Andromeda 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 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 Andromeda 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 Remove loose parts (capillaries and capillary lid) before transport as they may damage the measurement optics.

3. The Andromeda system

3.1. General

3.1.1. Intended use

The Andromeda system provides fast and highly sensitive detection and quantification of thermal stability of proteins 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 Safety requirements for electrical equipment for measurement, control and laboratory use. Part 1 General Requirements
- IEC 61010-2-010:2014 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 Electrical equipment for measurement, control and laboratory use – EMC requirements. Immunity test requirements for equipment intended to be used in a basic electromagnetic environment.
- 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 - Limitation of voltage changes, voltage fluctuations and flicker in public low-voltage supply systems, for equipment with rated current ≤16 A per phase and not subject to conditional connection.

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 conformity symbol.

Figure 1: Identification labels for Andromeda.

3.2. Technical information

3.2.1. Technical specifications

Electricity

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 Andromeda Series instrument	24 VDC, 10 A
Pollution degree	2

Environmental

Operating temperature	15–30 °C (indoor use only)
Storage temperature	-20–30 °C
Humidity	0–80 %, non-condensing
Operating altitude	max 2000 m

Andromeda dimensions

Width	35 cm (13.8”)
Height	55 cm (21.6”)
Depth	60 cm (23.6”)
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

Temperature control

Temperature control range	15 °C–95 °C (at 25 °C)
Precision of temperature control	± 0.1 °C

Noise level

Noise level of Andromeda instrument	max 64 dB(A)

3.2.2. Connections for input and output

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

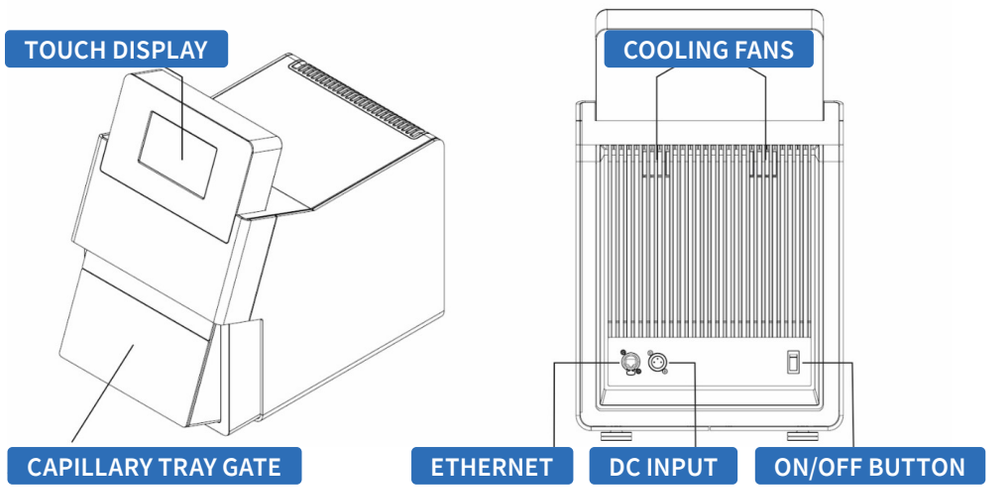

Figure 2: Connections on the Andromeda device.

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

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 Andromeda are registered trademarks of NanoTemper Technologies GmbH in the United States of America and other countries.
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. Andromeda setup

The Andromeda 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 Andromeda system package contains the following items:

Item	Description
Andromeda system	-
User manual	This user manual
Cables	Power cord and external power supply, Network cable for connection to control notebook
Control Notebook	Control Notebook for Andromeda system

4.2. Unpacking

The Andromeda 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 Andromeda system to power by plugging in the power supply cable. Connect the Andromeda system to the control PC using the ethernet connection at the back of the instrument. The system starts upon switching the power switch at the back.

4.4. Cleaning

The Andromeda 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 70 % ethanol.

4.5. Software updates

Software updates of the embedded system or the control software can only be performed by instructed NanoTemper Technologies personnel and is part of regular maintenance visits.

4.6. Installation requirements

To ensure operation safety, observe the following conditions:

- Only operate the Andromeda instrument with the supplied external power supply.
- Only connect the external power supply of the Andromeda to an electrical socket containing a protective conductor terminal.
- Ensure that the power plug of the external power supply is easily accessible. The Andromeda instrument has to be installed in a way that it does not hinder the access to the external power supply and its power plug.
- Only operate the instrument with the delivered notebook.
- The maximum noise level of the instrument is 64 dB(A). Only operate the instrument in an environment where this noise level is appropriate.
- Operate the instrument in a temperature range of 15 – 30 °C.
- Operate the instrument under non-condensing conditions and a dewpoint below 18 °C.
- Operate the instrument in an atmospheric pressure range of 800 – 1060 hPa.
- Do not operate the Andromeda instrument under conditions which pose a risk of explosion, implosion or the release of gases.
- Avoid strong magnetic fields and sources of high frequency. The instrument may not function properly when near a strong magnetic field or high frequency source.
- Avoid vibrations from vacuum pumps, centrifuges, electric motors, processing equipment and machine tools.
- Avoid dust and corrosive gas. Do not install the instrument where it may by exposed to dust, especially in locations exposed to outside air or ventilation outlets.
- To clean the instrument, only use water or 70 % ethanol.
- Do not install the instrument in a location where it may be exposed to direct sunlight.
- Install the instrument in a horizontal and stable position (this includes a table, bench or desk upon which the instrument is installed).
- Ensure that no air conditioner blows air directly onto the instrument. This may prevent stable measurements.
- Install the instrument in a location that allows easy access for maintenance.

NOTE The above conditions do not guarantee optimal performance of this instrument.

4.7. Installation and connecting cables

4.7.1. Preparation

Prepare a table which can bear a weight of about 50 kg (110 lbs) and has a free area of 80 cm (width, 31.5”) x 80 cm (depth, 31.5”). Put the Andromeda instrument and the control notebook on this free area.

CAUTION The weight of the Andromeda instrument is approximately 30 kg (66 lbs), do not move the instrument alone (two persons required for transport/ movement). If you move the instrument alone it poses a risk of personal injury or damage to the instrument.

4.7.2. Connecting the power supply and the Andromeda

Confirm that the power switch of the Andromeda instrument is off (power switch is at the back).

WARNING! Only operate the Andromeda instrument with the external power supply provided. Only use the supplied cables and plugs. If not doing so, you risk electric shock and fire.

Connect the external power supply to the electrical socket. Then connect the power supply to the Andromeda instrument.

CAUTION Ensure that the power plug of the external power supply is well accessible. The Andromeda instrument must be installed in a way that it does not hinder the access to the external power supply and its power plug.

Connect the Andromeda instrument to the control notebook by using the supplied network cable.

Switch on the Andromeda and the notebook.

5. Troubleshooting

5.1. Disconnect from control PC

In case of a disconnect between instrument and control PC the instrument would still complete the ongoing measurement. This is indicated by the touch display. Please do not switch off the instrument in this state. Wait until the measurement is complete and reestablish the connection. The Andromeda Control software will then obtain the measured data from the internal instrument PC.

5.2. Restarting Andromeda

In case the system freezes, wait one minute. If it does not un-freeze, use the switch at the back of the system to switch off the instrument. Wait 30 seconds for complete shutdown, then restart the instrument. The system will start up again automatically.

5.3. Customer support

In case of any issues not described in this user manual, please don’t hesitate to contact NanoTemper Technologies customer support at

nanotempertech.com/support.

6. Patents and intellectual property

Andromeda is patent protected, especially by the following patents, US20180284002A1, US20150137005A1 including their application and registration in different other countries.

7. Maintenance and disposal

7.1. Cleaning

Switch off the instrument and remove the power plug of the external power supply from the electrical socket. Only use a dry cloth or a cloth dampened with water or 70 % ethanol for cleaning the instrument.

CAUTION Only use water or 70 % ethanol to clean the instrument.

7.2. Repackaging for transport

The Andromeda 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 packaging material and shipment.

7.3. 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.

7.4. 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.

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AN.CONTROL

Software Manual

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NanoTemper® Technologies' Andromeda series instruments detect changes in the fluorescence of GFP fusion and labelled His-tagged proteins over a wide range of temperatures. The instruments can be used to induce thermal unfolding of proteins and to determine thermal unfolding transition temperatures.

The AN.Control software is dedicated to running and analyzing thermal unfolding experiments on Andromeda and Andromeda Plex instruments.

1. Technical Information

1.1. System Requirements

If the necessary licenses have been purchased, AN.Control software can be installed on additional computers for convenient data analysis. The computers have to meet the following requirements:

Operating system:	Windows 7 64 Bit or higher
CPU:	Intel Core i5 or better
RAM:	8 GB or more
Hard disk:	20 GB or more free disk space available
Display resolution:	1600 x 900 or better
Software:	Microsoft .NET 4.6.2 framework (included in installer of AN.Control software)
Operating system language:	English or German

An external computer mouse is necessary to access all software features.

1.2. Backward Compatibility

Measurements collected with all previous versions of AN.Control software are compatible with this version.

NanoTemper Technologies provides support for all current and previous versions of AN.Control.

2. Home Screen and General Usage

To perform a new measurement, start the AN.Control software, which will show the *Project* home screen. Click *Create New Project* to enter a file name and location. The file will be saved in .anc format. Optionally, enter relevant information into the *Project Name* and *Comments* fields.

Alternatively, previous project files can be loaded by clicking *Browse to analyze* previous experiments or to add additional measurements. Recently loaded files are listed chronologically on the right.

Use the *Save Changes* button at any time to save modifications of the file. An asterisk in the software title bar indicates unsaved changes in the open file. Closing the software will trigger a dialogue box asking whether you want to save the changes.

After an initial setup step (Choose Excitation Color), there are three tabs that guide the user through running and analyzing Andromeda measurements:

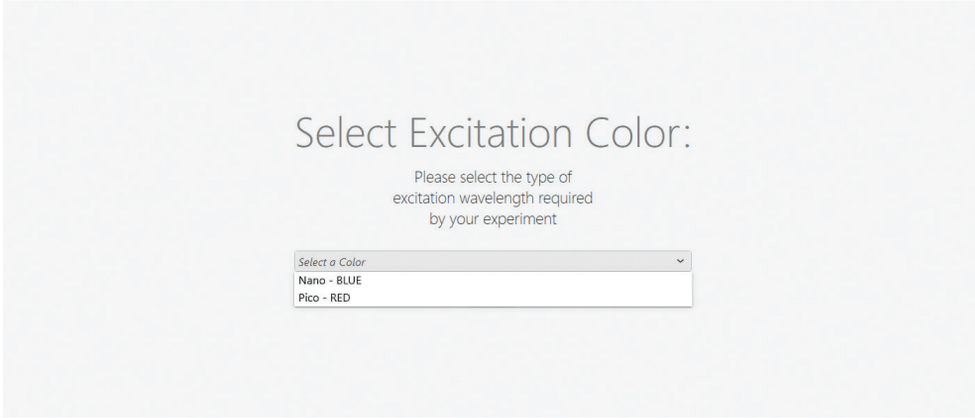
1. Discovery Scan
2. Melting Scan
3. Annotations and Results

Proceed from 1 to 3 to set up, run and analyze an Andromeda measurement. As soon as the measurement is started, navigate between all three tabs freely to re-analyze, modify or annotate. More details on each tab follow below.

The keyboard shortcut ctrl + z will undo any action, while ctrl + y will redo.

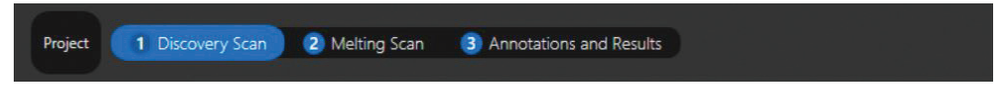
Any graph displayed in the software can be exported by clicking the Export button. Options are to copy the graph to the clipboard, to save it as an image (.png or .svg file formats), or to export the raw data needed to recreate the graph in third-party software (.xlsx file format).

3. Choose Excitation Color

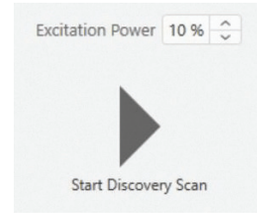


After creating a new project or loading a previous project file, first select the appropriate Excitation Color based on your sample type. For His-tagged proteins, choose the RED fluorescence channel to perform thermal unfolding after labeling your samples with the Andromeda His-Tag Labeling Kit (Cat. No. AN-030002). For GFP fusion proteins, choose the BLUE channel to perform thermal unfolding without labeling your samples.

4. Discovery Scan



Once a fluorescence channel has been selected, perform a *Discovery Scan* (1) to determine optimal settings for the unfolding experiment. You can vary the excitation power between 1 % and 100 %. Re-scan with different excitation power settings if necessary.



The discovery scan is used to detect the fluorescence intensity and position of each capillary along the entire length of the capillary tray. Each capillary will be visible as a peak, which represent the fluorescence intensity 670 nm in the RED channel or 510 nm in the BLUE channel, respectively. (Figure 1).

The upper and lower limits of detection are highlighted by dotted red lines in the discovery scan profile. The upper limit is 20,000 fluorescence counts in the RED channel and 2,000 fluorescence counts in the BLUE channel, respectively (Peak Fluorescence, meaning the height of the capillary peak). Please note that the lower detection limit is dynamically adjusted to the excitation power settings and thus may vary between experiments with different settings.

Zoom into the graph using the scrolling function of the mouse wheel. Holding down shift or ctrl while scrolling will zoom horizontally or vertically, respectively. Holding the mouse wheel also allows to move the graph. To reset the view, click *Zoom Extent*.

Note: *Try to aim for a fluorescence signal of approximately 10,000 counts in the RED channel, and of approximately 1,500 counts in the BLUE channel. With a final 1 nM concentration of RED-tris-NTA 2nd Generation dye in the sample, 10,000 counts should be obtained at 50 – 70 % excitation power in the RED channel.*

Note: *Photobleaching effects are negligible even at high excitation powers due to the rapid on-the-fly measurement mode.*

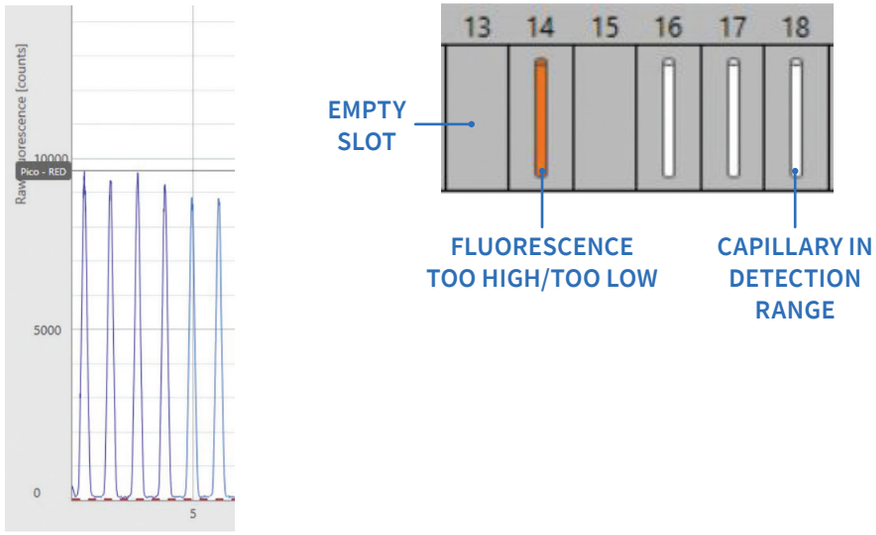
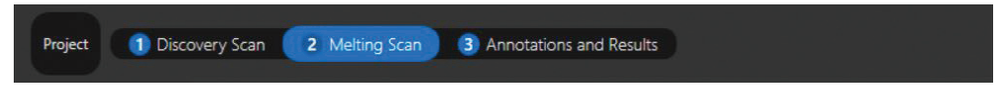


Figure 1: Discovery Scan. The discovery scan provides information about the fluorescence intensities of the samples, and is used to determine optimal measurement settings and the positions of occupied capillary slots. (Left) The fluorescence intensities of the 670 nm or 510 nm fluorescence channels are displayed as single peaks for each capillary. Clicking on a specific peak or capillary will highlight this capillary's fluorescence at the chosen wavelength, and also display its values for Peak Fluorescence and Integrated Fluorescence on the right of the screen. Each peaks should be between the upper and lower detection limits (dotted red lines). Capillaries with fluorescence intensities within the dynamic detection range are colored white; capillaries with too high or too low fluorescence intensities are colored red.

5. Melting Scan

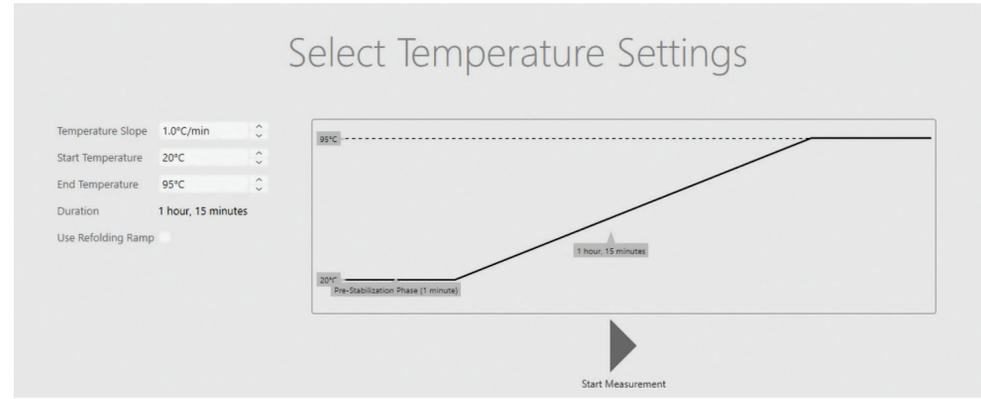


Once the optimal excitation power settings are determined, continue with the *Melting Scan* (2). The AN.Control software automatically identifies the capillaries for unfolding analysis from the discovery scan. By default, only capillaries that lie in the dynamic detection range are pre-selected for the unfolding experiment. However, you can also add capillaries that are marked in red.

Note: *You can multi-select capillaries using shift or ctrl + left click.*

Next, define the "Start" and "End" temperature for the thermal unfolding experiment, and set the "Temperature Ramp" to a heating rate of 7.0 °C/min.

Start the thermal unfolding experiment by pressing "*Start Measurement*". After a pre-melting phase in which the start-temperature is reached and the system is equilibrated, continuous scanning of the selected capillaries proceeds while a thermal ramp is applied. The thermal unfolding of proteins in each capillary can be followed in real time.



Once the final temperature is reached, unfolding transition midpoints will be automatically calculated by the software and can be displayed in the

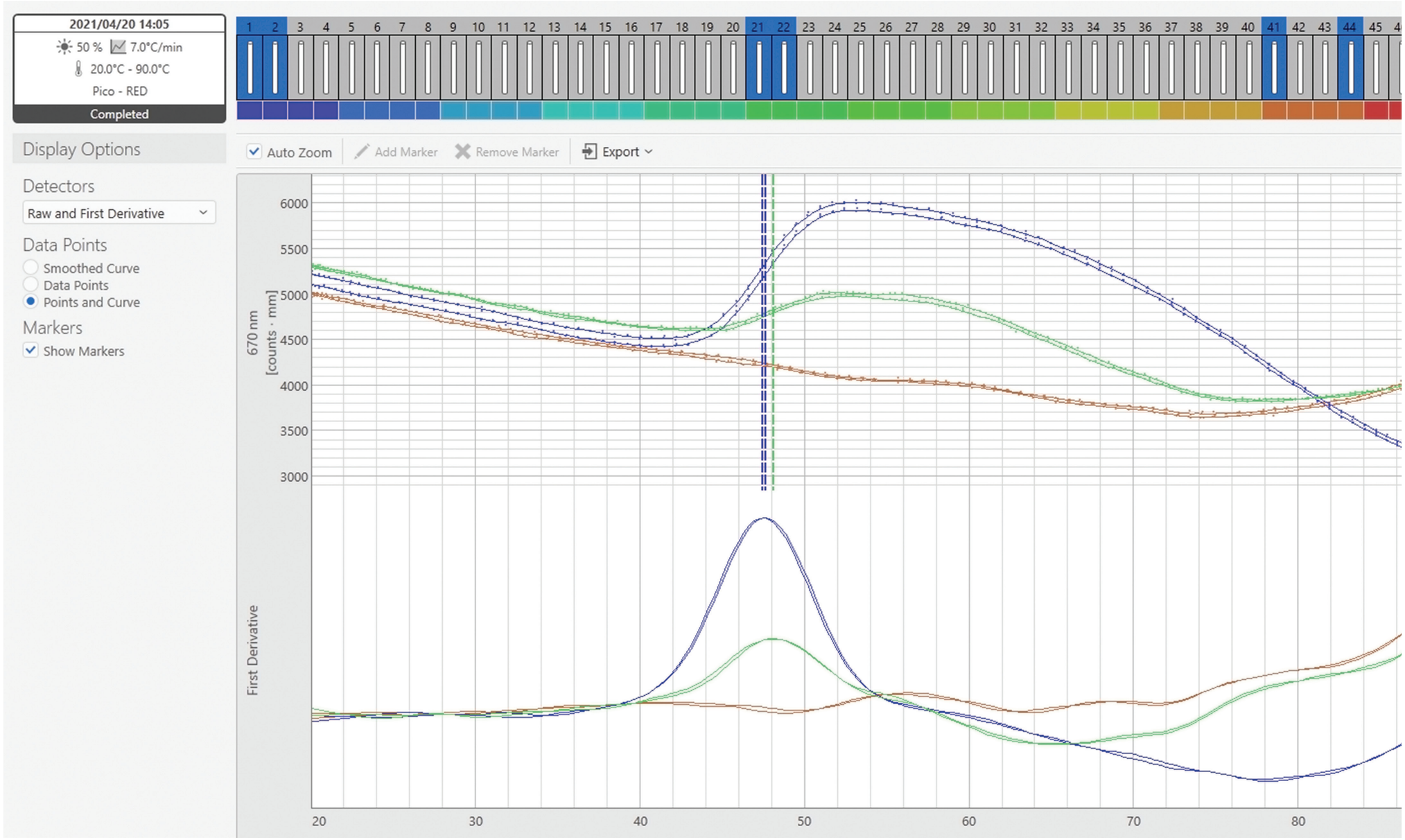
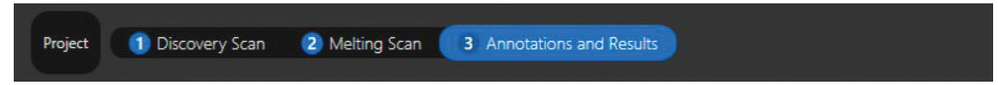


Figure 2: The Melting Scan window after an experiment has been completed.

melting scan window (see Figure 2). The window will also display the unfolding curves and, if selected, the respective first derivative of the curves. In the first derivative view, each local maximum or minimum corresponds to an inflection temperature (T_i, also known as Inflection Point, IP). Inflection temperatures can be manually added or removed by clicking the *Add / Remove Marker* buttons. Colored buttons below the capillaries can be used to manually change the color of each curve.

The calculated unfolding transition temperatures as well as manually annotated markers will be added to the annotations table. Raw fluorescence and processed data (raw fluorescence and first derivative values) as well as the corresponding calculated unfolding transition midpoints and annotations can be exported in Excel™ format using the *Export button.* Images can be exported as .svg or .png files.

6. Annotations and Results



It is not required to enter sample annotations prior to the experiment. Annotations for each capillary can be entered at any time while the melting scan is running or after it is finished. Annotations can either be entered by simple copy-and-paste from a spreadsheet software like Excel™ or manually. Columns can be added or removed by using the *Create New Column* and *Remove Column* buttons on the right side of the screen.

Annotations can be entered into multiple fields simultaneously after multi-selection, and subsequently sorted in ascending or descending manner by left-clicking on the column header.

Note: *Multi-selections of capillaries applied in the* Melting Scan *or* Annotations and Results *tabs persist after switching tabs.*

Capillaries can be selected and a single color can be assigned by clicking on the rectangle next to "*Single Color*". Furthermore, capillaries can be colored by categories or gradients after selecting the cells that are to be used for categorizing. The colors will directly translate into the melting curves in the *Melting Scan* window. Color assignment will only apply to selected fields. Multi-selection by using shift / ctrl + left click is possible.

The annotations table includes separate columns for unfolding transition midpoints (inflection points, IP also called T_i) and manually annotated points (M).

The thermal stability of a given protein is typically described by the thermal unfolding transition midpoint, also called inflection point (IP) or T_i (inflection temperature) [°C]. It is determined automatically by the AN.Control software via the derivative of the curve. This method circumvents the subjective determination of baseline levels and also allows for the determination of multiple unfolding transition midpoints, e.g. for more complex multi-domain proteins.

Annotation tables can be exported in Excel™ format (.xlsx).

7. Data Export

Each measurement and analysis performed can be exported to be used in third-party software. Throughout this software manual, different export options are mentioned. This section aims to give an overview regarding the file format and the content of each export.

7.1. Discovery Scan

The Discovery Scan tab contains three export options.

Clicking on *Export Raw Data* will create an Excel™ file (.xlsx) containing two sheets. The first sheet gives the major information for each peak, whereas the second one contains the information to reproduce the graph.

The *Copy Chart to Clipboard* button will copy the graph displayed so you can paste it into another software.

The *Export Graph* button will create an image file (.png or .svg) of the zoom extended view displayed by the software.

7.2. Melting Scan

The export button in this tab will display four options.

The *Copy Chart to Clipboard* button will copy the graph exactly how it is displayed in the software (for example only showing selected capillaries, or showing both raw data and first derivative) so you can paste it into another software.

The *Export Graph* button will create an image file (.png or .svg) of the graph as described above.

Clicking on *Export Raw Data* will create an Excel™ file containing four sheets. The first sheet gives an overview of all capillaries contained in the run, including unfolding transition midpoints, unfolding onsets and manually set markers. The other sheets contain the fluorescence at 670 nm or 510 nm as a function of temperature for all capillaries, respectively.

Using *Export Processed Data* will create the exact same Excel™ file but including also the data of the first derivative for each fluorescence channel as separate sheets.

7.3. Annotations and Results

The export option is located at the bottom right of the screen and it will create an Excel™ file containing the table displayed in this tab.

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AN.STABILITY ANALYSIS

Software Manual

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NanoTemper® Technologies' Andromeda series instruments detect changes in the fluorescence of GFP fusion and labelled His-tagged proteins over a wide range of temperatures. The instruments can be used to induce thermal unfolding of proteins and to determine thermal unfolding transition temperatures.

The AN.Stability Analysis software is dedicated to analyzing thermal unfolding data acquired with AN.Control software on Andromeda and Andromeda Plex instruments. Combine any data you generated to quickly pinpoint trends and provide statistical relevance from your merged replicate data sets.

1. Technical Information

1.1. System Requirements

If the necessary licenses have been purchased, AN.Stability Analysis software can be installed on additional computers for convenient data analysis. The computers have to meet the following requirements:

Operating system:	Windows 7 64 Bit or higher
CPU:	Intel Core i5 or better
RAM:	8 GB or more
Hard disk:	20 GB or more free disk space available
Display resolution:	1600 x 900 or better
Software:	Microsoft .NET 4.6.2 framework (included in installer of AN.Stability Analysis software)
Operating system language:	English or German

An external computer mouse is necessary to access all software features.

1.2. Compatibility

Measurements collected with all versions of AN.Control are compatible with AN.Stability Analysis.

1.3. Installation and License Activation

The software can be installed on any computer and will automatically start in trial mode, when opened for the first time. The trial version offers full functionality and has to be activated with a license key purchased from NanoTemper Technologies after 30 days.

To activate the license, navigate to the main menu and select *Licensing Status.* Please follow the instructions provided. One software license is valid for one account per computer and licenses can be transferred between computers. To transfer a license, please deactivate it first via the *Licensing Status* dialogue and reactivate it on the new computer.

2. Term Definitions

Merge Set: A series of replicates of samples from a single or multiple data files collected with AN.Control, with identical temperature settings. Data within one Merge Set will be averaged and the standard deviation calculated. The software will additionally calculate and display error bands in the charts.

Analysis: A complete dataset consisting of any number of Merge Sets or single Andromeda samples from single or multiple data files, for comprehensive and systematic analysis and direct comparison. The analysis can be saved as an analysis file (.ana) at any time.

Raw Data: All data recorded by the Andromeda instrument: fluorescence traces of single wavelengths at 670 nm (RED) or 510 nm (BLUE). When exporting Raw Data, this also includes derived values such as first derivative and standard deviations for each data point.

3. Home Screen and General Layout

To perform a new analysis, start the AN.Stability Analysis software, which will show the analysis home screen. Click on *AN.Control Analysis* to create an analysis from data recorded with AN.Control software. Select the respective .anc file(s). The analysis will be saved in .ana format.

Alternatively, previous analysis files can be loaded by clicking *Browse*. Recently loaded files are listed chronologically on the right.

Use the *Save Analysis* button at any time to save modifications of the analysis file. An asterisk (*) in the title bar indicates unsaved changes in the current analysis. Closing the software will trigger a dialogue box asking whether you want to save the changes.

All major functions of the AN.Stability Analysis software are organized in 4 different sections (see Figure 1):

1. Sidebar
2. Menu Bar
3. Data Table
4. Charts and Display Options

Additional buttons in the menu bar are:

5. Quick saving of the analysis file
6. Support

Alerts will be displayed on the top right of the main window.

The keyboard shortcut ctrl + z will undo any action, while ctrl + y will redo.

4. Sidebar

4.1 Analysis Data

4.2 Global References

4.3 Analysis Templates

4.4 Batch Export

Figure 2: Functionalities located in the sidebar.

4.1. Analysis Data

The *Analysis Data* menu allows adding, removing and filtering data files from AN.Control.

When creating a new analysis, enter an analysis name and optionally add comments, e.g. purpose of the analysis, assay conditions etc.

To add more data to the analysis use the *Add Files* function to browse folders and select single or multiple .anc files. Runs of the selected file(s) will appear in the list of loaded files in the Analysis Data window. Click on to show only samples from the selected datafile or experiment. Click on  to remove single experiments or a whole datafile from the Analysis. Alternatively, clicking Remove Excluded Samples will delete single samples or Merge Sets from the Analysis after they have been marked as excluded in the data table.

Note: *Files can also be added to the analysis directly from Windows Explorer by using drag-and-drop.*

4.2. Global References

The *Global References* menu allows saving of a reference currently assigned in the Analysis. Saved global references can be reused later on any other data set by clicking Assign. References are saved locally on the PC's hard drive. To access them, open Windows Explorer and type %localappdata%\Nanotemper in the address bar. Reference files can be copied and moved to different PCs or users and reused there.

For more information on setting references, see 5.1.

4.3. Analysis Templates

Analysis Templates consist of Key Parameters and Regions of Interest and can be saved from the current data set. Reuse them later on any other data set. When an analysis template is applied to a data set:

- The selected Key Parameters are automatically plotted for all samples in the data set.
- Regions of Interest are applied and evaluated on the selected samples or on all samples in the data set, depending on whether the *Apply to all samples* checkbox is checked.

Analysis Templates are saved locally on the PC's hard drive. To access them, open Windows Explorer and type %localappdata%\Nanotemper in the address bar. Analysis template files can be copied and moved to different PCs or users and reused there.

4.4. Batch Export

Export selected samples or the complete Analysis Set. Please refer to chapter 8 for details on available options.

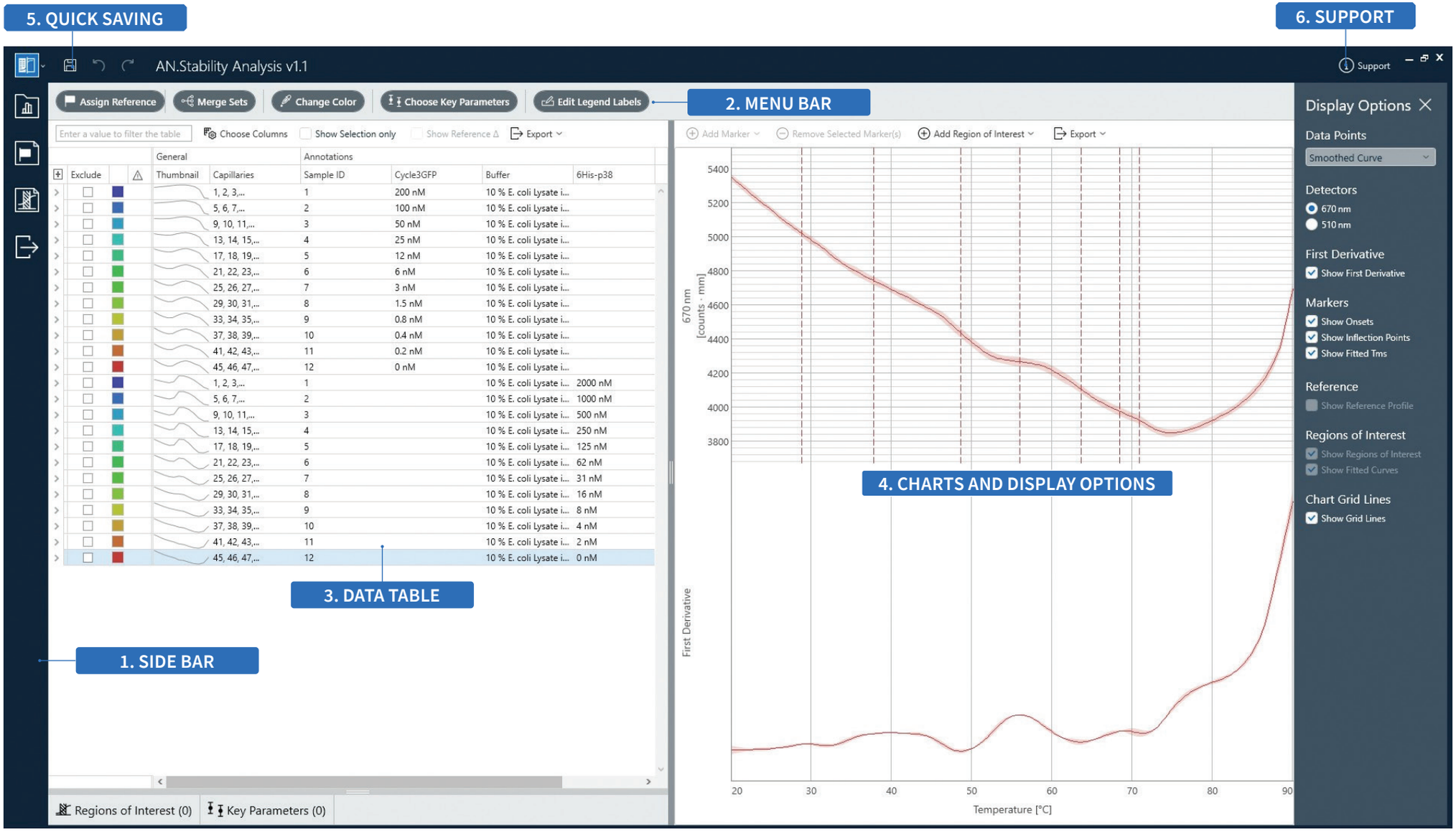


Figure 1: General layout of AN.Stability Analysis.

5. Menu Bar

5.1. Assign Reference

AN.Stability Analysis allows to set any sample in the current dataset as a reference. This can be either a Merge Set or a single capillary. Clicking the *Assign/Unassign Reference* button will assign/unassign the currently selected sample as reference. Only one reference can be used at a time.

The reference will be highlighted in the data table and its unfolding profile is shown in the charts in gray (if selected to be shown via the Display Options).

References can also be saved as Global Reference (see 4.2.) and reused later on any other dataset.

5.2. Merge Sets

AN.Stability Analysis automatically detects and merges replicates by their annotation(s). By default, merging is and all available annotation columns are used to identify replicates. Merging is case-sensitive. Which annotation columns in the original data file are used for merging can be customized by checking or unchecking the respective checkbox in the *Merge Sets* menu. Unchecking *Enable merging of replicates* will undo all merging operations.

Note: *Merging can alternatively be performed by dragging and dropping individual capillaries or Merge Sets in the data table.*

Note: *Samples acquired with different heating rates will not be merged, even if otherwise fully identical.*

5.3. Change Color

The coloring scheme assigned and stored in the .anc files is imported into AN.Stability Analysis. Coloring can be changed and customized for individual capillaries, Merge Sets or the complete Analysis. Options are Single color, Gradient color, or Category color.

To color by gradients requires selecting multiple samples. The colors will directly translate into the curves displayed in the charts and Key Parameter plots. Color assignment will only apply to selected samples.

Multi-selection by using shift / ctrl + left click is possible.

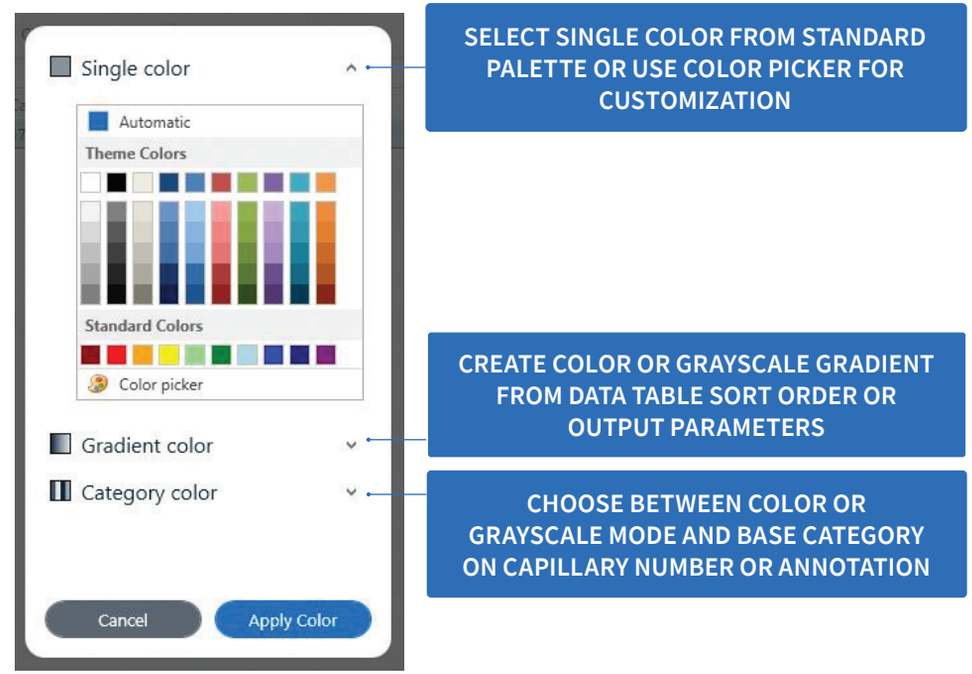
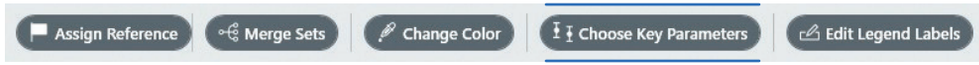


Figure 3: Coloring options.

5.4. Choose Key Parameters



Individual output parameters can be plotted for the whole Analysis with the Key Parameter feature. Select from the list the parameters to be plotted by checking the respective checkboxes. The plot(s) will be displayed below the data table and contain all samples of the current Analysis, for which the parameter is available (see Figure 4). Capillaries or Merge Sets, for which the *Exclude* checkbox is checked in the data table, will not be shown in the Key Parameter plot. Each sample is depicted as data point and error bars indicate the standard deviation for average values from Merge Sets. Click on a data point to select it in the data table.

Key parameter plots can be exported to Excel™ (.xlsx) or as image (.png).

5.5. Edit Legend Labels

Define in the *Edit Legend Labels* menu, which annotations should be used in the legends of the charts; for exporting or to identify samples in the Key Parameter plot. Legend labels will by default be set to automatic mode, but you can switch to manual mode to select other label types.

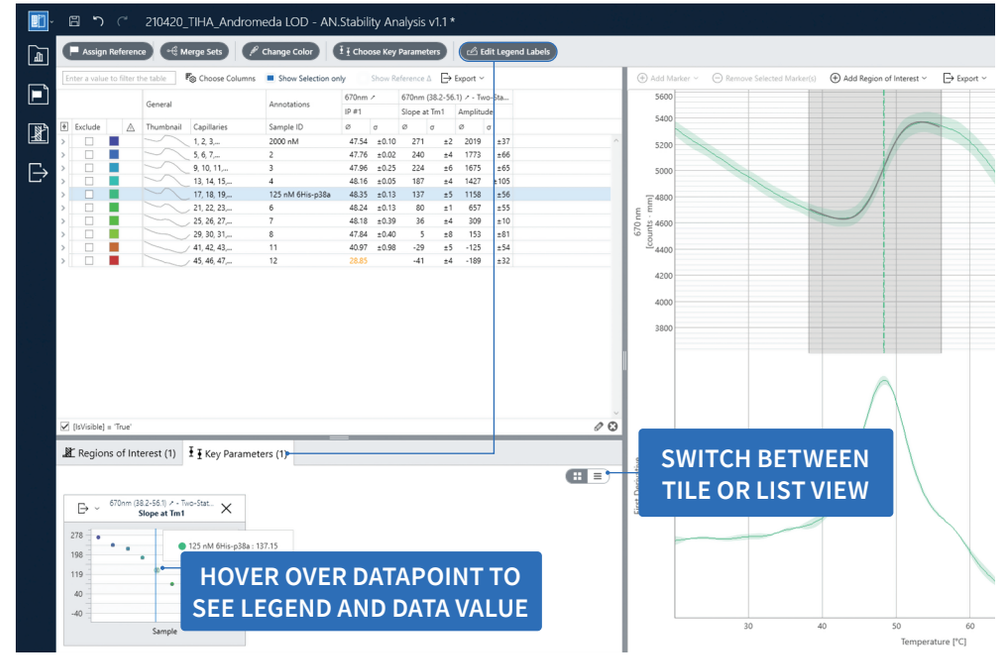


Figure 4: Additional functionalities in Key Parameter plots.

6. Data Table

6.1. Sample Organization and Merge Sets

Replicates will be automatically combined into Merge Sets upon loading data into AN.Stability Analysis. Merge Sets are shown as a tree structure in the data table (see Figure 5). For each Merge Set, average values and standard deviations are calculated automatically.

Merging behavior can be customized via the *Merge Sets* function in the menu bar (see 5.2.). Alternatively, samples can be moved between different Merge Sets by drag and drop.

Navigate between samples by using arrow keys up/down, and right/left to expand and collapse Merge Sets.

Single samples, replicates or whole Merge Sets can be excluded from the analysis by checking the *Exclude* checkbox (see Figure 5). It is possible to completely delete these samples from the Analysis via the Analysis Data tab located in the sidebar (see 4.1.)

Multi-selection of several rows by using shift / ctrl + left click is possible.

All available annotation columns will be loaded from the AN.Control file. Annotations can be manually changed by double-clicking into the respective cell. Content from single annotation cells may be copied to other annotation cells using copy-and-paste after selection. Changing an annotation for a Merge Set will change this annotation for replicates contained in the Merge Set. Additional annotation columns can be added by using *Choose Columns* (see following section).

6.2. Customizing Views

The data table includes separate groups of columns for general information on the experimental settings, annotations, and output parameters. The order of columns and column groups can be changed by dragging and dropping the respective header cell into a new position. Pin column groups to the left or right end of the table by right clicking on a group header cell and selecting *Left* or *Right* in the *Fixed Style* menu.

Sort values by clicking on the column header. The next to the header indicates the sorting order (ascending, descending, or off). Alternatively, rightclicking on column header opens a menu with sorting options.

Hovering over a header cell makes  visible. Click it to open the filter menu and filter samples based on selected values. The  will stay visible in the header after a filter has been applied to a particular column (see Figure 5).

Which columns are shown can be customized via *Choose Columns* by checking/unchecking the respective checkbox in front of the column name or by clicking *Select All*. The list contains all columns available for the current analysis and only a subset will be shown by default. You can always return to this default by clicking *Reset to default*.

6.3. Output Parameters

Output parameter columns are grouped based on the data they originate from: 670 nm, 510 nm, Regions of Interest/Fits.

Arrows in the column headers indicate that the values were determined during a heating ramp  .

Values for inflection points as well as manual markers are imported from the AN.Control file. They are sorted into respective columns based on temperature. Manual markers are shown in italics.

The data table can be exported in Excel™ format (.xlsx) or csv.

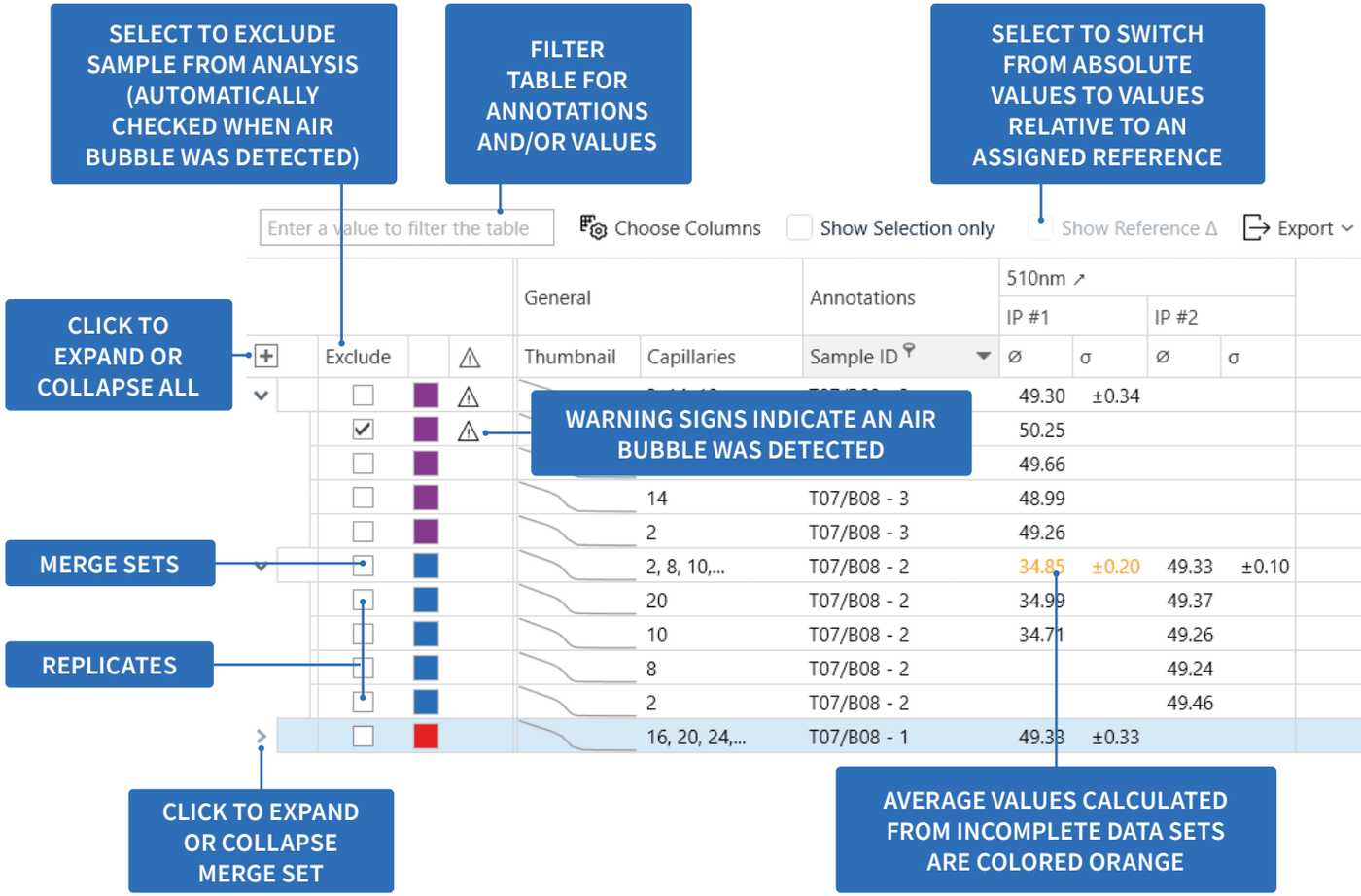


Figure 5: Functionalities in the data table.

7. Charts and Display Options

All samples selected in the data table will be displayed in the charts panel. Multi-select samples by using shift or ctrl + left click.

For Merge Sets, the software will automatically calculate and display error bands.

Zoom into the graph using the scrolling function of the mouse wheel. Holding the mouse wheel also allows to move the graph horizontally. To reset the view, click *Reset Zoom*.

The calculated inflection points (IP) can be displayed in the charts by checking *Show Inflection Points* (see Figure 6).

Shown are also the unfolding profiles and, if selected, the respective first derivative of the curves. In the first derivative view, each local maximum or minimum corresponds to an Inflection Point. Inflection points can be manually added or removed by clicking the *Add/Remove Marker* buttons. Removing markers on the Merge Set will remove the respective value from all replicates within this set. Manual markers can only be added to individual replicates, but not to Merge Sets.

Inflection points can be added to the 670 nm and 510 nm plots. The manually annotated markers will be added in italics to the data table and sorted into the respective column based on their temperature value.

Any chart displayed in the charts panel can be exported by clicking the *Export* button . Options are to save it as an image (.png) or vector graphics (.pdf).

7.1. Regions of Interest

Data from AN.Control (670 nm or 510 nm) can additionally be fitted to a thermodynamic model.

To perform a fit, first select which type of fit (Two-state fit or Three-state fit) you want to use via the *Add Region of Interest* menu and then select the respective region by clicking at the start and end point in the plot (see Figure 6). The calculation of fit parameters will then be carried out automatically.

Calculated values will be added as separate columns in the data table.

All defined Regions of Interest will appear in a separate panel below the data table next to the Key Parameters .

RoI can be saved as Analysis Templates (see 4.3.).

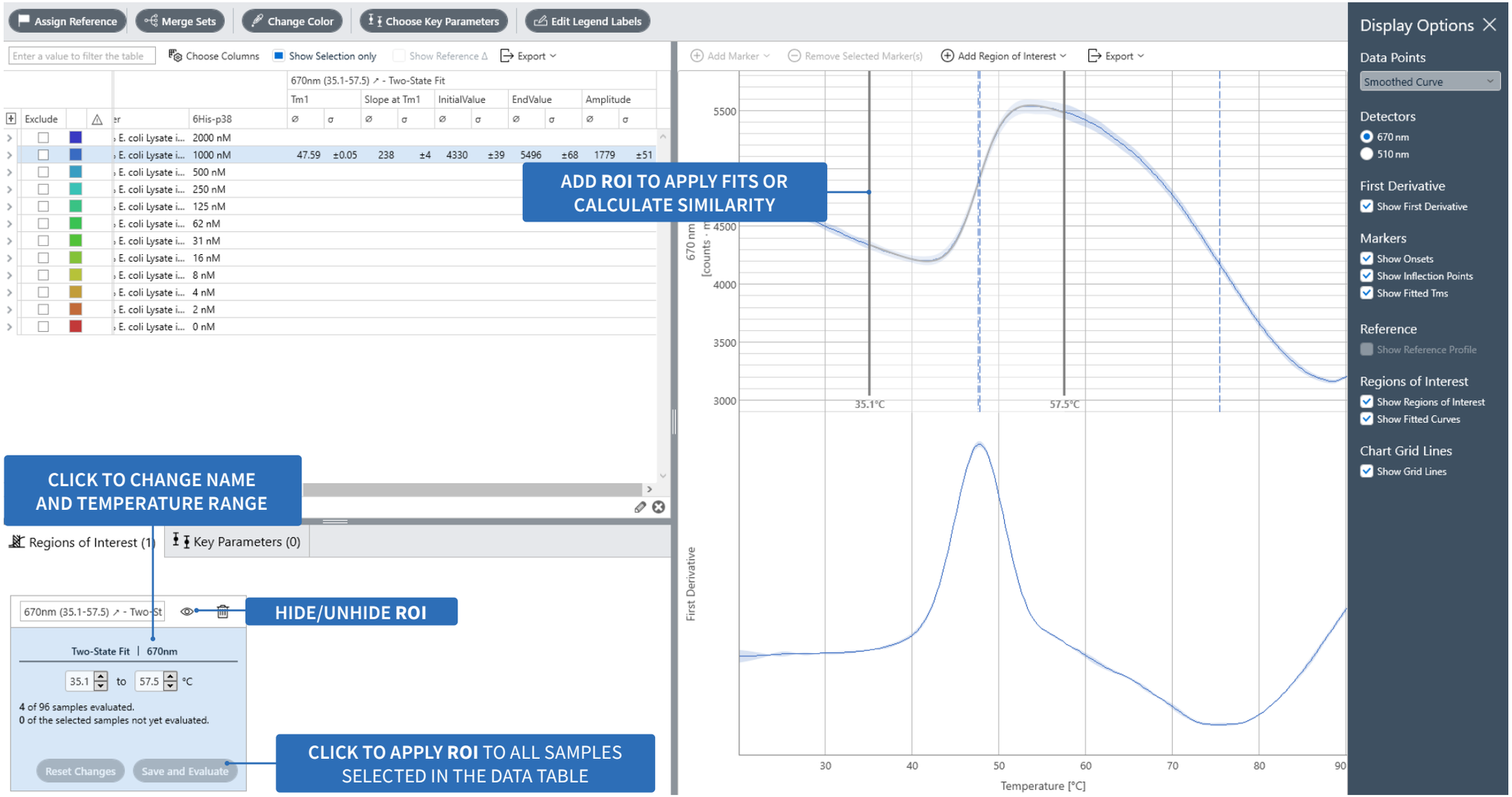


Figure 6: Display options in the charts panel and working with Regions of Interest (RoI).

8. Save and Data Export

Each measurement and analysis performed can be exported to be used in third-party software. Throughout this software manual, different export options are mentioned. This section aims to provide an overview regarding the available file formats.

Access the export menu(s) via the button and select from the following options:

- The data table can be exported in Excel™ format (.xlsx) or as .csv
- Graphs can be exported as an image (.png) or vector graphics (.pdf)
- Key Parameter plots can be exported to Excel™ format (.xlsx) or as image (.png)

The *Edit Legend Labels* menu lets you define which annotations should be used in the legends of the charts (see 5.5.). These labels will be used for exporting or to identify samples in the Key Parameter plot.

Additionally, the full Analysis or selected parts can be exported via the *Batch Export* function in the sidebar (see 4.4.). This includes processed as well as Raw Data. Batch export settings can be saved as presets.

V01_2021-06-16

ANDROMEDA

Labeling Kit Manual

Table of contents

1. Purpose and Contents of This Kit

For site-specific, purification-free labeling of His-tagged proteins with RED-tris-NTA 2nd Generation dye.

For use in Andromeda, sufficient for 5,000 single-point measurements

Content and Storage

Andromeda His-Tag Labeling Kit is shipped at room temperature.

Each kit contains material sufficient for 5,000 single-point measurements.

2* 125 pmol RED-tris-NTA 2nd Generation dye [store at -20 °C]

1* 2 mL 5 x PBS-T (for 10 mL 1 x PBS with 0.05 % Tween 20) [store at 20 °C]

1* 1200 pmol His6 Control Peptide [store at -20 °C]

Expiry date: see kit cover

Additional Material Required

Variable speed benchtop microcentrifuge

1.5 mL microcentrifuge collection tubes

0.2 mL PCR tubes

ddH2O

2. Protein Labeling Procedure

The Andromeda His-Tag Labeling Kit provides convenient means for the site-specific, purification-free labeling of His-tagged proteins with our fluorescent RED-tris-NTA 2nd Generation dye.

This kit can be used for the labeling of any protein carrying a polyhistidine-tag (His-Tag) and contains material sufficient for 5,000 single-point Andromeda measurements. Labeling can be completed in 30 min, no removal of excess dye is required.

The RED-tris-NTA 2nd Generation dye can bind efficiently to His-tags which contain at least six histidines with a K_d in the single digit nM range. By following this protocol, optimized for nearly 100 % binding of dye to His-tagged proteins eliminates an additional purification step and ensures the best results. RED-tris-NTA 2nd Generation dye shows fluorescence excitation and emission maxima at approximately 650 nm and 670 nm, respectively.

2.1. Buffer Compatibility

The protocol describes our best labeling practice for the Andromeda and Andromeda Plex instruments.

His-tags are common protein tags which are routinely used for affinity purification. The His-tag labeling strategy is highly specific, requires only nM concentrations of His-tagged proteins and no dye-removal step. Labeling can be carried out even with unpurified samples, in cell lysate or other complex bioliquids. Moreover, His-tag labeling is robust towards a variety of common storage and assay buffer components. Concentration limits for some buffer components which might interfere with the labeling reaction are listed in Table 1.

We recommend using phosphate-buffered saline (PBS) or alternatively HEPES buffer and a pH in the range of 7-8 for the labeling reaction. As the affinity between the dye and the His-tag decreases significantly at a pH below 7, these conditions are not advised.

Component	Maximum allowed concentration
Histidine	1 mM
Imidazole	1 mM
EDTA, EGTA, other chelating agents	0.05 mM
TCEP*	0.5 mM
DTT	5 mM
β-mercaptoethanol	1 mM
GSH	10 mM
GTP, GDP	1 mM
AMP, ADP, ATP	5 mM
Glycerol	10 %
Co^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}	preloaded protein only**
Polyhistidine-tagged species	none
pH <7	not recommended
SDS	not recommended

Table 1: List of common buffer components and their maximum allowed concentration

* NanoTemper Technologies recommends avoiding the use of TCEP with the red dyes in general.

** Co^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+} ions compete for the binding with RED-tris-NTA 2nd Generation dye. Because of that reason only low nanomolar concentrations of listed ions are tolerated.

2.2. Step-by-step Instructions

Step A

Lysate Preparation

Lysates prepared from any organism (bacteria, yeast, insect or mammalian cells) can be used.

Perform lysis of cells expressing His-tagged protein using any method of your choice, e.g. sonication, homogenization, chemical or enzymatic lysis. Choose a lysis buffer that is compatible with protein labeling, e.g. PBS or HEPES buffer with a pH > 7.0 and a salt concentration of at least 150 mM. Please refer to Table 1 for a list of incompatible substances.

NOTE: Make sure to perform a centrifugation step to remove cell debris and protein aggregates (e.g. 10 minutes at 20,000 x g and 4 °C) before subjecting your samples to protein labeling (Step B).

NOTE: Make sure that your clarified cell extract does not contain any genomic DNA as this might interfere with protein labeling. Disrupt genomic DNA before protein labeling, e.g. by sonication or enzymatic treatment.

Step B

Protein Labeling

The following protocol describes the labeling procedure for one experiment, with replacement of scientists' interaction buffer of choice for Phosphate-buffered Saline + 0.05 % Tween 20 (PBS-T) if choosing to use an alternate buffer system. Volumes can be scaled up- or down when needed. We recommend sample preparation in PCR tubes or in 96- or 384-well multi-well plates with non-binding surface.

1. Add 8.0 mL ddH2O to the vial containing 5 x PBS-T to obtain 1 x PBS-T.
2. Suspend the content of one vial of RED-tris-NTA 2nd Generation dye (125 pmol) in 50 µL of PBS-T to obtain a 2.5 µM dye solution.
3. Prepare a 25 nM dye solution by mixing 2 µL of dye (2.5 µM) and 198 µL PBS-T.
4. Predilute the lysate sample containing the His-tagged protein by mixing 10 µL of lysate and 90 µL PBS-T.
5. Mix 48 µL of prediluted lysate sample containing the His-tagged protein with 2 µL of dye (25 nM). Mix well by carefully pipetting the sample up and down 4-5 times (do not vortex!).
6. Incubate for 30 minutes at room temperature in the dark.
7. Mix all samples again by carefully pipetting each sample up and down 4-5 times.
8. The protein is now labeled and ready to be subjected to the thermal unfolding assay. The amount of each sample (50 µL) is sufficient to run four technical replicates.

Step C

Andromeda Assay

Load the sample into capillaries and measure the samples in the RED channel. Recommended settings are 60 % Excitation Power. At the final dye concentration of 1 nM, the expected fluorescence intensity at 60 % Excitation Power is around 10,000 counts on Andromeda and Andromeda Plex.

3. FAQ

1. Can I perform thermal shift assays in crude lysates with Andromeda?

Yes, thermal shift assays to assess binding competency of the His-tagged protein target can be performed even in crude mixtures with Andromeda. Make sure to add ligand at a concentration that is sufficient to saturate the target protein, and to always include proper negative controls (e.g. lysate + ligand solvent) and avoid any buffer mismatches between samples.

2. My protein requires a divalent cation or co-factor for proper function. May I add it to PBS-T buffer during labeling?

Yes, cofactors required for the protein function can be added directly to the PBS-T buffer. Please check Table 1 for limitations. Divalent ions like Ca^{2+} cannot be added to PBS as this will result in precipitation. Alternatively, HEPES buffered saline can be used as labeling and assay buffer.

3. The cells expressing my protein of interest were lysed in a buffer which is not compatible with this kit. What are the alternatives?

In this case a predilution of the lysate samples with a compatible buffer (e.g. PBS-T) is recommended. Please refer to Table 1 for a list of incompatible substances and their maximum allowed concentrations, and predilute the lysate samples accordingly. Alternatively, lyse your cells in a buffer that is compatible with this kit.

4. Can I store the RED-tris-NTA 2nd Generation dye and Control Peptide solution?

Yes, the solutions may be stored for about 8 weeks. We recommend freezing the stock solutions in 510 µL aliquots at -20 °C.

5. How can I be sure that I am really seeing my His-tagged target protein in the thermal unfolding assay?

Labeling His-tagged protein with RED-tris-NTA 2nd Generation dye is possible even in crude mixtures due to the dye's high affinity and specificity for His-tagged proteins. However, in complex environments like cell lysate, the use of appropriate controls is always advisable to control for unspecific interactions. It is recommended to always prepare and run a negative control sample alongside, e.g. cell lysates prepared from untransformed cells or cells harboring an empty plasmid backbone. If applicable, the purified His-tagged protein in buffer is an ideal positive control sample that can be run alongside.

4. Troubleshooting

Observation	Possible Reasons	Remedy
No unfolding detectable, or unfolding signal barely distinguishable from noise	His-tagged protein is not or very poorly expressed	Skip predilution of lysate sample (Step B3.)
	His-tagged protein is unfolded	Optimize protein expression conditions
	Suboptimal buffer conditions for protein labeling	Review assay buffer for incompatibilities with His-tag labeling (pH, EDTA, metal ions, etc.)
	His-tag is not well accessible	Perform control experiment with purified protein and RED-tris-NTA dye in lysis buffer
No thermal shift observable upon ligand addition	Target protein has no binding activity	Perform control experiment with purified protein and ligand in lysis buffer
	Ligand is being scavenged by other proteins in the sample	Increase ligand concentration

5. Safety Information

Dye

Hazard statements

H315	Causes skin irritation.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.

Precautionary statements

P264	Wash hands thoroughly after handling.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P302+P352	IF ON SKIN: Wash with plenty of water.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313	If eye irritation persists: Get medical advice/attention.

For more information, please consult the respective Safety Data Sheets (SDS). SDS are available from NanoTemper Technologies upon request.

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6. Contact

6.1. Technical Support

Please get in touch with us for specific questions concerning the product performance. Find all kinds of supporting material or submit a support case via our Explorer Community:

nanotempertech.force.com/explore

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NanoTemper cares for the future of our planet, therefore we want to do our best to protect it and reduce the environmental footprint of our products.

*This product manual is printed in Germany, using **EU Ecolabel approved paper from certified FSC® responsible forestry.***

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