

Microscale Thermophoresis (MST) – Nanotemper Monolith NT.115

In a nutshell: Challenging biomolecular interactions measured in solution using microliter-volume samples in a capillary format. A wide range of ligands and affinities can be analyzed in various buffer conditions, including cell lysates and plasma.

Services: Biophysics Facility offers MST as an open-access instrument. First-time users must complete a short training session before gaining access to the instrument reservation calendar. Training includes the K_D determination of a standard molecular interaction. During the MST training, you will use our pipetting robot to quickly prepare the dilution series for titration.

Location: Building 50, room 3123.

Description: MST measures a wide range of interactions under close-to-native conditions. It can be used to study the interactions of proteins, DNA, RNA, small ligands, and other molecular systems. The versatility of this method is achieved by detecting changes in the molecular charge, size, and hydration shell of molecules induced by ligand binding. In the MST instrument a focused infrared light beam is used to generate precise, microscopic temperature gradients (with a 1°C to 5°C temperature differential) within thin-glass capillaries filled with a sample prepared in a buffer or bio-liquid of choice. The motion of molecules along the temperature gradient is monitored by the fluorescence of a probe attached to one of the molecules. Both fluorescent dyes and fluorescent proteins (e.g. GFP) can be used as trackers.

Typical applications:

- measurements of a wide range of biomolecular interactions in solution
- binding measurements in challenging conditions, including cell extract, plasma, and other bio-liquids.

Basic instrument specifications:

- Detection wavelengths:
 - Blue LED: Ex:470(±10)/Em:520(±10) nm (labels: NT495, FITC, GFP, YFP)
 - Red LED: Ex:625(±25)/Em:684(±6) nm (labels: NT647, Cy5, Alexa647)
- Affinity range: 1 nM – 500 mM
- MW range: 10 Da to 100 MDa
- Capillary (sample) volume 5 µL

- Number of capillaries: A maximum of 16 capillaries can fit on the instrument tray; 12 to 16 capillaries are used for a binding titration.
- Concentration of the labeled molecules: From 10 nM to 1 mM. See below for more information.
- Temperature range: 22-40 °C
- Time required for a single analysis: Up to 30 minutes for one 16-point titration measurement and data analysis. More time is needed for sample preparation and control experiments. Tedious serial dilutions can be automated using the Biophysics Core Facility pipetting robot.

Sample requirements and recommended buffers:

- The typical concentration of the labeled molecules is 50 nM, with a minimum of 10 nM. The minimum concentration depends on the type of fluorescent tag and labeling efficiency. The concentration of the labeled compound should always be lower than the expected dissociation constant value. When using NTA-conjugated dye for HIS-tag labeling, the minimum label concentration should be at least 50 nM to avoid dissociation of the label.
- To prepare samples with a 50 nM concentration of labeled molecules, a 100 nM stock is required. This stock concentration should always be twice the concentration used in the titration.
- The minimum ligand stock concentration should be 20x the expected dissociation constant value. If the K_D is unknown, we recommend a 20 μ M ligand stock concentration.
- MST can use a variety of buffers, all of which are supplemented with 0.02-0.05% v/v Tween-20 to prevent molecules from adhering to the capillary surface.

Minimum sample amount:

- A minimum ligand stock volume of 20 μ L is required for a single titration, and an additional 15 μ L is needed for control experiments. Control experiments can often be performed once for a series of titrations.
- A minimum of 160 μ L of the labeled molecule stock is required for a single 16-point titration, and an additional 120 μ L is needed for control experiments. Control experiments can often be performed once for a series of titrations.
- The volumes listed above assume a preparation of 20 μ L titration samples. These samples require a minimum pipetting volume of 10 μ L, and can be prepared with high precision and accuracy manually or using our pipetting robot, which is a recommended method. Sample volume can be reduced with some loss of data quality.
- Minimum amount of proteins required for labeling:
 - NHS (primary amines) labeling: Protein concentration: 2 - 20 μ M, minimum stock volume 40 - 100 μ L. The absolute sample minimum: 100 μ L at 2 μ M. IMPORTANT: The minimum amounts listed above are often lower than what is required to positively confirm the protein concentration or measure the extent of labeling on a

spectrophotometer. For reliable result, use a sample concentration high enough to perform accurate absorption measurements.

- NTA HIS-tag labeling: The protein stock concentration of 200 nM at a 100 μ L volume. This yields a minimum amount sufficient for a single titration and a single control experiment set. If necessary, an additional amount may be required to check the effect of the ligand on the His-tag labeling stability.

Consumables:

- We stock both the standard and premium (low adhesion) MST capillaries.
- We also stock the NHS (primary amines - Lysine residues) and HIS-tag labeling reagents and labeling kits.

Consumables are provided at the manufacturer's prices.