

Quick Start Protocol for Sample Preparation for Microscale Thermophoresis

Prerequisite and general remarks:

- One substrate should be fluorescently labeled. Make sure, that there isn't any not reacted, free dye in your sample (it is best to use the NanoTemper labeling kit manual).

- Prepare at least 20 μ l of sample per capillary to avoid pipetting error and changes in concentration due to sample evaporation. You will need approx. 5 μ l to fill a capillary.

- The concentration of the labeled molecule is always kept constant. The unlabeled compound is varied in concentration.

- Do not use less than 10 capillaries for an analysis of interaction.

- The observed fluorescence counts of the labeled sample in the respective dilution should be about 80-1500 fluorescence counts when measured with the Monolith NT.115.

- The concentration of the labeled molecule should be in the range of the expected KD or less.

- The concentration of the titrated compound should vary from about 20x to 0,02x the expected KD.

1. Spin down the stocks of the fluorescently labeled molecule and the molecule you intend to titrate in a serial dilution (5min, 13000 rpm in a table top centrifuge).
2. Check the buffer composition of the labeled compound and the compound that is titrated.
3. Prepare a stock solution of the fluorescently labeled molecule at twice the concentration you want to use in the experiment. Prepare at least 200 μ l (10 μ l x 16 Capillaries + 1-5 Test measurements). Use the buffer you want to use for determining the affinity.
4. Mix the stock well and test the fluorescence intensity in a capillary in the NT.115 instrument and test the sample as suggested in the manual at 3.2 and 3.3.
5. Put the stock on ice and protect it from light.
6. Prepare small 16 micro reaction tubes, best suited are tubes with a volume of 100 μ l or less. Do not use reaction tubes with higher volume for volumes of 20 μ l or less. This may lead to strong evaporation and may change concentration due to a low volume to surface ratio in the tube. Number them from 1 through 16.
7. Fill 20 μ l of the highest concentration you intend to use in the first micro reaction tube number 1.
8. Fill 10 μ l of the buffer you want to use for dilution into the micro reaction tubes 2 to 16. Please note: The buffer in tube number one and the buffer in the other tubes must be the same. Otherwise you get a gradient in salt, DMSO, glycerol or other additives. This interferes with the thermophoretic measurement.
9. Transfer 10 μ l of tube number one to tube number two and mix very well by pipetting up and down several times.
10. Change the tip of the pipet and transfer 10 μ l in the next tube. Repeat this 15 times and remove 10 μ l from tube number 16 after mixing.

11. Mix 10 μ l of fluorescently labeled sample with the 10 μ l of the titrated compound and mix well by pipetting up and down several times. Please note that you only have half the concentration you initially had. (It is more precise to prepare 15 μ l of the titrated sample, transfer 10 μ l to fresh tubes and then mix with 10 μ l of the labeled molecule)
12. Incubate the sample at conditions of your choice before filling it into the capillaries. Incubation temperature and time can differ between different molecules. In most cases 30-60 minutes incubation at room temperature are sufficient.

NOTE:

You should mix small volumes by pipetting up and down. Vortexing does not work for small volumes.