

# Cat Nr: MO-C032 Monolith NT<sup>™</sup> Control Kit LabelFree

MicroScale Thermophoresis Grade

# Control Kit for performing standard biomolecule interaction experiments using Monolith instruments including LabelFree detectors

Content and Storage	Monolith NT™ Control Kit LabelFree is shipped at room temperature. Store at -20 °C upon arrival	
	Each kit contains material sufficient for 5 reactions	
	4 x Vial A (32 μg Protein)	
	4 x Vial B (25 μg Ligand)	
	4 x Vial C (500 µl of 1 x Buffer)	
	100 x 200 μl Tubes	
	Expiry date: see kit cover	
INTRODUCTION	Control Kits contain biomolecules and a protocol for performing standard biomolecule interaction experiments using Monolith NT™ instruments. These kits are recommended	
	<ul> <li>when using Monolith instruments for the first time</li> <li>for training new lab employees</li> <li>for monitoring the correct performance of Monolith instruments</li> </ul>	

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#### **SAMPLE PREPARATION PROTOCOL**

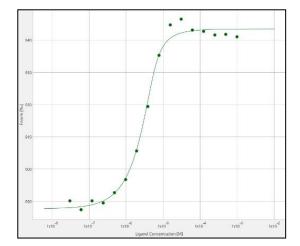
Please check the control software version available on your Monolith. For Monolith instruments equipped with MO.Control software, follow the instructions below. For Monolith instruments equipped with NTControl, please see next page for instructions.

#### **PROTOCOL FOR USE WITH MO.CONTROL SOFTWARE**

- 1. Add 25 µl of vial C (Buffer) to the vial B (Ligand) to dissolve the ligand. Let the solution sit for 5 minutes, then mix thoroughly by pipetting up and down. This will yield a 2 mM solution.
- Add 200 µl of vial C (Buffer) to vial A (Protein) to dissolve the protein. Let the solution sit for 5 minutes, then mix thoroughly by pipetting up and down. DO NOT VORTEX. Make sure that the protein is dissolved completely. This will yield a 10 µM solution.
- 3. Start a Binding Affinity experiment. Fill in the Plan page as shown below. If supported by your Monolith instrument, it is also possible to use the auto-detect function for determining the optimal LED power setting. Then follow the instructions given by the software to run the experiment. On the Instructions page, the buffer is referred to as ligand buffer.

🚯 Target		🔶 Ligand	
Protein	~ ?	Ligand	~ ?
Concentration of stock solution	10 µM ~ ?	Estimated Kd	optional µM ~ ?
Concentration in this assay	5µМ 🧨 ?	Concentration of stock solution	2000 µM ~ ?
Assay buffer		Ligand in organic solvent like DMSO	?
MST Buffer including 0.1% Pluronic	?	Ligand buffer in this assay	50.0%
fl. carillani		Highest concentration in this assay	1000µM 🧷 ?
Capillary     Monolith NT.Labelfree Zero Background Standard Treated Capillary (Z002     System settings	) ~ ?		
- Excitation Power			
✓ Auto-detect 10% ↔ ? Medium ∽	?		

4. The interaction should show a  $K_d$  of around 1.5  $\mu$ M.





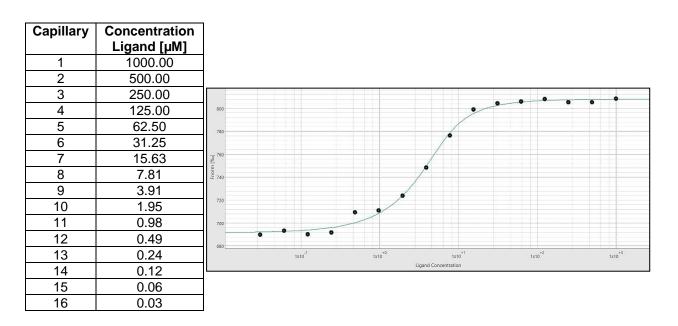
#### **PROTOCOL FOR USE WITH NTCONTROL SOFTWARE**

- 1. Add 25 µl of vial C (Buffer) to the vial B (Ligand) to dissolve the ligand. Let the solution sit for 5 minutes, then mix thoroughly by pipetting up and down. This will yield a 2 mM solution.
- 2. Prepare 16 reaction tubes. Add 20 µl from vial B (dissolved Ligand) to the first tube. Add 10 µl of vial C (Buffer) to the other 15 tubes. Transfer 10 µl with a clean tip from the first tube to the second tube and mix well by pipetting up and down. With a clean tip transfer 10 µl from the second tube to the third tube and mix well. Continue this 1-fold serial dilution until tube number 16. Remove and discard 10 µl from tube 16.
- 3. Add 200 μl of vial C (Buffer) to vial A (Protein) to dissolve the protein. Let the solution sit for 5 minutes, then mix thoroughly by pipetting up and down. DO NOT VORTEX. Make sure that the protein is dissolved completely. This will yield a 10 μM solution. Add 10 μl from vial A (dissolved Protein) to each tube containing Ligand dilution. Mix well by pipetting.
- 4. Load the instrument

Fill 16 Monolith<sup>™</sup> NT.LabelFree Zero Background Standard Treated Capillaries with the prepared solutions and transfer the capillaries to the tray. Load the tray into the Monolith and start the measurement using the settings in the table below. First time users can find more detailed information on how to perform measurements in the instrument user manuals and starting guides.

LED power	10 %
MST power	40 %

5. Fit the data using the Kd model and fix the target concentration (TargetConc / Fluo. Conc.) to 5  $\mu$ M. The interaction should show a  $K_d$  of around 1.5  $\mu$ M.





# Contact

## **TECHNICAL SUPPORT**

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

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