Control Kit RED

For Dianthus, Monolith and NT.Automated Instruments with a RED Detector

Cat# MO-C030

Content and storage

Control Kit RED is shipped at room temperature
Store at -20 °C upon arrival

Each kit contains material sufficient for 4 reactions

4x Vial A (40 nM AptamerCy5 RED)
4x Vial B (10 mM AMP)
4x Vial C (1x Reaction Buffer)
100x 200µl Tubes

Expiry date: see kit cover

Introduction

Control Kits contain biomolecules and a protocol for performing standard biomolecular interaction experiments using Dianthus, Monolith or NT.Automated instruments. These kits are recommended

- when using Dianthus, Monolith or NT.Automated instruments for the first time
- for training new lab employees
- for monitoring the correct performance of instruments

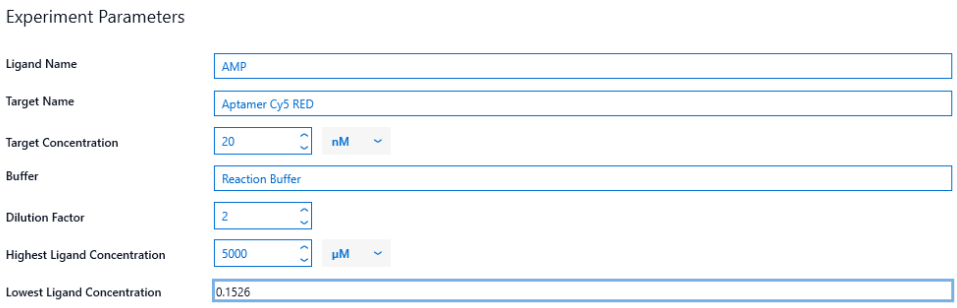
Sample preparation protocols

Please find below protocols for Dianthus, Monolith and NT.Automated. Make sure to use the correct protocol for your device.

DIANTHUS

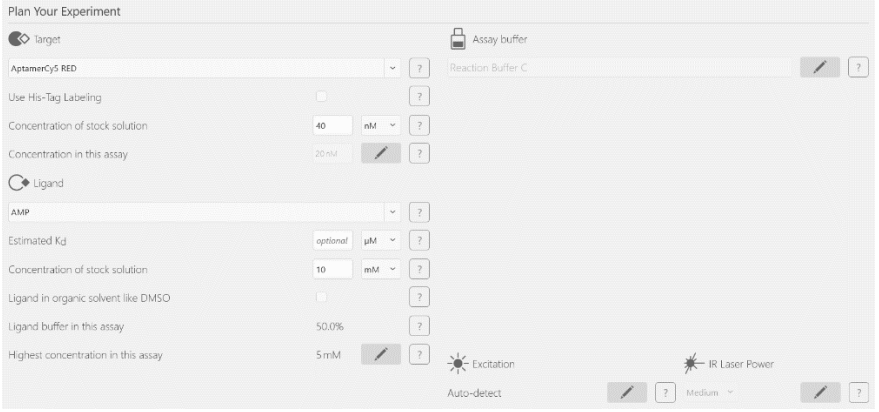
Protocol for use with DI.Control software

1. Unpack the kit and thaw the solutions. Spin each vial for a few seconds to ensure that any liquid stuck in the cap is spun to the bottom of the vial and not lost upon opening. Mix each vial well by pipetting.
2. Prepare a Dianthus plate with 16 unused wells in a row or column. Add 20 µl of vial B (AMP) to the first well. Add 10 µl of vial C (Reaction Buffer) to the other 15 wells. Transfer 10 µl of AMP from the first well to the second well and mix by pipetting up and down. Next, transfer 10 µl from the second well to the third well and mix. Continue this factor 2 serial dilution until well number 16. Remove and discard 10 µl from well 16.
3. Add 10 µl of vial A (AptamerCy5 RED) solution to each tube containing AMP dilution. Mix well by pipetting.
4. Spin Dianthus plate for 15 seconds in a plate centrifuge to remove air bubbles from the wells.
5. Start a Binding Affinity experiment. Set the temperature control to 25 °C. Fill in the experiment parameters as shown below. Use the auto-detect function for determining the optimal LED power setting. Only for Dianthus NT.23Pico and Dianthus NT.23PicoDuo instruments, set the Pico Sensitivity setting to off.
6. Analyze the resulting data in Dianthus Screening Analysis using a Hill model to fit. The interaction should show an EC50 ~30 µM.



MONOLITH and NT.AUTOMATED
Protocol for use with MO.Control software

1. Unpack the kit and thaw the solutions. Spin each vial for a few seconds to ensure that any liquid stuck in the cap is at the bottom of the vial and not lost upon opening. Mix each vial well by pipetting.
2. If you are using Monolith Pico or NT.Automated with a Pico detector: transfer 20 µl from vial A (AptamerCy5 RED) into a clean tube and add 180 µl of vial C (Reaction Buffer) to obtain a 4 nM stock solution of the target. In step 3, please replace 40 nM with 4 nM in the Plan page for the concentration of stock solution and change the target concentration in this assay from 20 nM to 2 nM. Mix well, then proceed with step 3.
3. Start a Binding Affinity experiment. Set the temperature control to 25 °C. Fill in the Plan page as shown below. Verify that the IR Laser Power (MST Power) is set to 'Medium'. If supported by your Monolith instrument, use the auto-detect function for determining the optimal Excitation setting. Otherwise, set the excitation to 20%. Then follow the instructions provided by the software to run the experiment.
   On the Instructions page, the reaction buffer is referred to as ligand buffer.
   For sample loading, Monolith Capillaries as well as Monolith Premium Capillaries can be used.
4. Analyze the resulting data by deselecting Automatic Mode in the Dose Response results details and choose the Hill model for fitting the data. The interaction should show an EC50 of ~30 µM.



MONOLITH

Protocol for use with NT.Control software

1. Unpack the kit and thaw the solutions. Spin each vial for a few seconds to ensure that any liquid stuck in the cap is at the bottom of the vial and not lost upon opening. Mix each vial well by pipetting.
2. Set the temperature control to 25 °C.
   If you are using a Monolith NT.115: Directly proceed to step 3.
   If you are using a Monolith NT.115Pico: Transfer 20 µl from vial A (AptamerCy5 RED) into a clean tube and add 180 µl of vial C (Reaction Buffer) to obtain a 4 nM solution of AptamerCy5 RED. Mix well, then proceed with step 3.
3. Prepare 16 reaction tubes. Add 20 µl of vial B (AMP) to the first tube. Add 10 µl of vial C (Reaction Buffer) to the other 15 tubes. Transfer 10 µl from the first tube to the second tube and mix well by pipetting up and down. Transfer 10 µl from the second tube to the third tube and mix well. Continue this serial dilution until tube number 16. Remove and discard 10 µl from tube 16.
4. Add 10 µl of the vial A (AptamerCy5 RED) solution prepared in step 3 to each tube containing AMP dilution. Mix well by pipetting.
5. Fill 16 Monolith Capillaries with the prepared solutions and transfer the capillaries to the capillary tray. Load the tray into the Monolith and start the measurement using the settings below.

LED power	20 %
MST power	40 %

6. Fit the data using the Hill model. The interaction should show an EC50 of ~30 µM.

Note: First time users can find more detailed information on how to perform measurements in the instrument user manuals and starting guides.

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