

C-Trap Reference Kit *Quick Protocol*

1. Cleaning and Passivation of the Microfluidics

1.1 Overnight incubation with Alkaline Cleaning Reagent

1. Fill all syringes with 2 ml Milli-Q water and flush all channels for 10 minutes at 2 bar.
2. Prepare a 10 ml solution of 1x Alkaline Cleaning reagent (ACR) by diluting 200 μ l of the 50x ACR solution with 9.8 ml Milli-Q water. This solution can be used in the next step.
3. Remove the remaining water (~1.5 ml) from the syringes and add 2 ml 1x ACR to each syringe. Flush all channels for 30 minutes at 2 bar.
4. Incubate overnight (no longer than 60 h).

1.2 Alkaline Cleaning Reagent Removal

1. Remove the remaining Alkaline Cleaning reagent (ACR) from the syringes (~500 μ l).
2. Replace the syringes on all channels with new ones. To ensure complete removal of ACR, avoid any residual liquid in the connector from splashing onto the syringe walls.
3. To wash, add 2 ml Milli-Q water to all syringes. Flush all channels for 10 minutes at 2 bar. Remove the remaining water (~1.5 ml).
4. Repeat the washing step (step 3) two more times. After the last wash, do not remove the remaining Milli-Q water.

1.3 Passivation with BSA 2%

1. Prepare 1 ml BSA 20 mg/ml in PBS to use immediately in the next step.
2. Remove the Milli-Q water from the syringe of channel 4 or 5 (protein channel).
3. Add 1 ml of freshly prepared BSA solution into the syringe connected to the designated protein channel. Keep Milli-Q water in the remaining channels.
4. Flush all channels for 2 minutes at 2 bar, followed by 10 minutes at 0.5 bar.
5. Ensure that at least 200 μ l of BSA solution has flushed through the protein channel.
6. Stop the flow and incubate for a minimum of 20 minutes (max 3 hours).

1.4 Equilibration in Binding Buffer

1. Prepare 20 ml 1x binding buffer by diluting the 10x binding buffer with Milli-Q water.
2. When ready to proceed with the experiment, empty the syringes of all channels.
3. Add 1 ml of buffer to all syringes. Flush all channels for 5-10 minutes at 0.5 bar. Remove the remaining binding buffer.
4. Repeat the equilibration step (step 2) two more times. After the last equilibration step, do not remove the remaining buffer.

2. DNA Tethering and Confocal Setup

2.1 Microfluidics Setup

Channel 1: Add 5 μl of streptavidin beads $\text{\O}4 - 4.9 \mu\text{m}$, 0.1% (w/v) to 500 μl of 1x binding buffer, mix by inverting the tube, and load it in the syringe of channel 1.

Channel 2: Add 2.5 μl of biotinylated DNA LacO to 500 μl of 1x binding buffer, mix by inverting the tube, and load it in the syringe of channel 2.

Channel 3: Load 500 μl of 1x binding buffer in the syringe of channel 3.

2.2 DNA Tethering

1. Set the eWLC to 37.8 kb contour length.
2. Flush channels 1, 2, and 3 at 0.3–0.5 bar, while keeping the protein channels (4 and 5) closed.
3. Trap two beads and calibrate.
4. Catch a single tether and confirm the right length with the eWLC model.

2.3 Confocal Setup

1. Set the pixel size to 50 nm and the pixel time to 0.2 ms.
2. In channel 3, stretch the DNA by moving Trap 1 until the force is 10 pN.
3. Position a 2D scan region to include both beads. Perform a single 2D scan with the red laser (638 nm) and confirm that both beads are within the optical section of the confocal scan.
4. Confirm that the ATTO 647N fluorophores of the DNA are in the confocal focal plane by moving “Trap1+2” along the z-axis.

3. Imaging LacI-ATTO 565 Binding in Kymographs

3.1 Flush in LacI-ATTO 565

1. Add 2 μl LacI-ATTO 565 to 300 μl 1x binding buffer, mix gently, and load in the protein channel.
2. Flush channels 1–3 + protein channel at 0.5 bar, and confirm LacI-ATTO 565 is equilibrating in the channel by starting an empty kymograph (no beads or DNA) with the 561 nm laser ON. Fluorescence should increase and reach a plateau within $\sim 3\text{--}5$ minutes.

3.1 Record Kymographs

1. Trap two beads, calibrate and zero the forces, and catch a single DNA tether.
2. Stretch the tether to ~ 10 pN.
3. Verify the tether is aligned with the confocal line scan using the ATTO 647N labels on the DNA.
4. Move the bead–DNA construct to the LacI channel.
5. Start recording a kymograph. Record 5–10 kymographs (5 minutes each), restarting a kymograph every 5 minutes with the same DNA tether.
6. In case a low binding rate is observed (less than 1 binding event per 10 seconds) or the DNA tether breaks, catch new beads and a new DNA tether, and flow channels 1–4 for 1–2 minutes before starting a new kymograph.