C–Trap Cleaning and Passivation Quick Protocol

1. Cleaning and Passivation

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 µl 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 µl).
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 and Casein

In preliminary C-Trap protein binding tests, assess the optimal passivating agent for your assay. This protocol describes how to passivate the microfluidics with 2% BSA and 1% Casein in parallel.

1. Remove the Milli-Q water from the syringes of channel 4 and 5 (protein channels).
2. Add 1 ml of 2% BSA and 1% Casein (both in PBS buffer) into the syringes of cahnnel 4 and 5, respectively. Keep Milli-Q water in the remaining channels.
3. Flush all channels for 2 minutes at 2 bar, followed by 10 minutes at 0.5 bar.
4. Ensure that at least 200 µl of BSA and Casein solutions have flushed through their respective channels.
5. Stop the flow and incubate for a minimum of 20 minutes (max 3 hours).

1.4 Equilibration

1. Prepare 30 ml of buffer of choice.
2. When ready to proceed with the experiment, empty the syringes of all channels.
3. Add 2 ml of buffer to all syringes. Flush all channels for 10 minutes at 0.5 bar. Remove the remaining binding buffer.
4. Repeat the equilibration step (step 3) two more times. After the last equilibration step, do not remove the remaining buffer.

2. Cleaning Protocol for DNA Intercalating Dyes

2.1 Overnight Incubation with Alkaline Cleaning Reagent

The following four steps are the same as those described in Section 1.1.

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 µl 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).

2.2 Removal of DNA Intercalating Dyes with Cleaning Reagent

Note: The Cleaning Reagent (CR) is not the same as the Alkaline Cleaning reagent (ACR) used for standard cleaning procedures without intercalator dyes.

1. After overnight incubation, remove the remaining Alkaline Cleaning Reagent (ACR) from the syringes (~500 µl).
2. Add 2 ml of Milli-Q water to all syringes. Flush all channels for 10 minutes at 2 bar.
3. Stop the flow and remove the remaining water (~1.5 ml).
4. Fill each syringe with 1 ml of Cleaning Reagent.
5. Flush 0.5 ml of Cleaning Reagent into the flow cell at 2 bar for 10 minutes.

2.3 Neutralizing with Reducing Agent

1. Remove the remaining Cleaning Reagent (~0.5 ml) from the syringes.
2. Install new syringes on all channels. Avoid splashing Cleaning Reagent onto the inner walls of the new syringes, as this helps ensure complete removal in later steps.
3. Fill each syringe with 2 ml of Milli-Q water and flush at least 0.5 ml Milli-Q water at 2 bar for ≥10 minutes.
4. Add 50 µl of Reducing Agent (30x) to the remaining of Milli-Q (≤1.5 ml) in each syringe and pipette to mix thoroughly. This results in a 1x solution of Reducing Agent.
5. Flush at least 1 ml of the neutralized solution through the system at 2 bar for 20 minutes.
6. Remove the remaining reducing reagent (≤0.5 ml) from the syringes.
7. To wash, add 2 ml of Milli-Q water to all syringes. Flush all channels for 10 minutes at 2 bar.
8. Stop the flow and remove the remaining water (~1.5 ml).
9. Repeat steps 3–4 two more times. During the final wash, keep the Milli-Q water in the system.

If required, proceed with passivation and equilibration described in Section 1.3 and 1.4, respectively.