C–Trap Cleaning and Passivation Protocol

Version 3

LUMICKS

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C–Trap Cleaning and Passivation Protocol

1. Description

The cleaning and passivation procedures described in this protocol rely on the materials provided by the **Beads & Cleaning Kit – Recommended**. The kit includes the Alkaline Cleaning Reagent (ACR), designed to thoroughly clean the microfluidics module of the C-Trap. ACR is highly efficient at eliminating biological materials such as DNA, proteins, lipids, and bioconjugated polystyrene beads. Its powerful cleaning action breaks down and lifts contaminants, leaving the microfluidics module clean and ready for reliable performance in sensitive single-molecule experiments.

Thanks to its superior performance compared to bleach-based cleaning reagents, ACR enables the use of high concentrations of passivating agents such as 2% bovine serum albumin (BSA) and 1% casein.

In addition to ACR, the kit contains two tubes of bioconjugated polystyrene beads, selectable via our webstore from a range of diameters and surface coatings (Streptavidin or Anti-Digoxigenin) to match your experimental needs (see section 2.1). If only the cleaning reagent is needed, the **Alkaline Cleaning Reagent (50x)** can also be purchased separately through our webstore.

Cleaning Reagent and Reducing Agent are available as optional add-ons with the kit in our webstore and can be used in combination with ACR to specifically remove DNA Intercalating Dyes. We also advise replacing the syringes of the microfluidics module after each cleaning cycle; syringes are also available as add-ons with the kit.

The reagents and beads provided with the kit are sufficient for up to 60 cleaning cycles and 60 experimental sessions on the C-Trap.

Although the kit does not include passivation materials, we provide recommendations for suitable products and their preparation.

Cleaning and passivation performance can be evaluated using the **C-Trap Reference Kit**, which includes a standardized DNA–protein interaction assay to assess microfluidics, workflow, and optics setup.

1.1 Important Points

- Cleaning with ACR requires overnight incubation.
- 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.
- The Cleaning Reagent (sodium hypochlorite, <5%) is different from the Alkaline Cleaning Reagent (ACR) included in the kit. The Cleaning Reagent is available as an add-on when purchasing the kit.
- The Alkaline Cleaning Reagent (ACR) is used for general cleaning of proteins, DNA, and biologically functionalized beads.
- The Cleaning Reagent is specifically required for the removal of DNA Intercalating Dyes such as YOYO-1 or SYTOX Orange.
- Cleaning and passivation performance can be evaluated using the C-Trap Reference Kit, which includes a standardized DNA–protein interaction assay to assess microfluidics, workflow, and optics setup.

2. Materials Supplied in the Kit

The Alkaline Cleaning Reagent (ACR), included in the kit, is sufficient for cleaning the microfluidics when working with proteins, DNA, and biologically coated polystyrene beads.

The kit also includes two tubes of bioconjugated polystyrene beads, which can be selected from a range of diameters and surface coatings (See section 2.1 for available options).

If DNA Intercalating Dyes have been used, the Cleaning Reagent (available as an add-on) must be used for effective dye removal. This should be used in combination with the Reducing Agent (also available as an add-on).

We also recommend replacing the syringes of the microfluidics module after each cleaning cycle. Syringes are available as an optional add-on.

Components	Supplied with Kit	Units	Total Volume	Storage Temperature
Alkaline cleaning reagent (50x)	Included	1	12 mL	RT
Selection of Polystyrene Beads	Included	1	See bead selection	+4 °C
Cleaning Reagent (1x)*	Available as add-on	1	300 mL	RT
Reducing Agent (30x)*	Available as add-on	1	15 mL	RT
Syringes	Available as add-on	100	-	RT

*Cleaning reagent and Reducing reagent are required for the removal of DNA Intercalating Dyes.

2.1 Bead Selection

Using the dropdown menu on our webstore, you can select different combinations of two tubes of beads. Each tube contains the volume listed below and is sufficient for up to 60 experiments in the C-Trap.

Bead Coating	Diameter (⌀)	Volume	Concentration (w/v)	Dilution factor
Streptavidin	1.0–1.4 µm	60 µL	1%	1:1000
	1.5–1.9 µm	60 µL	1%	1:1000
	4.0–4.9 µm	600 µL	0.5%	1:100
Anti-Digoxigenin	0.7–0.9 µm	600 µL	0.1%	1:100
	2.0–2.4 µm	600 µL	0.1%	1:100

2.2 Additional Materials (supplied by the user)

This protocol describes a passivation procedure in which microfluidic channels 4 and 5 are treated in parallel with 2% Bovine Serum Albumin (BSA) and 1% Casein, respectively.

Testing both agents side by side allows users to determine the optimal passivating agent for their specific protein binding assay in the C-Trap.

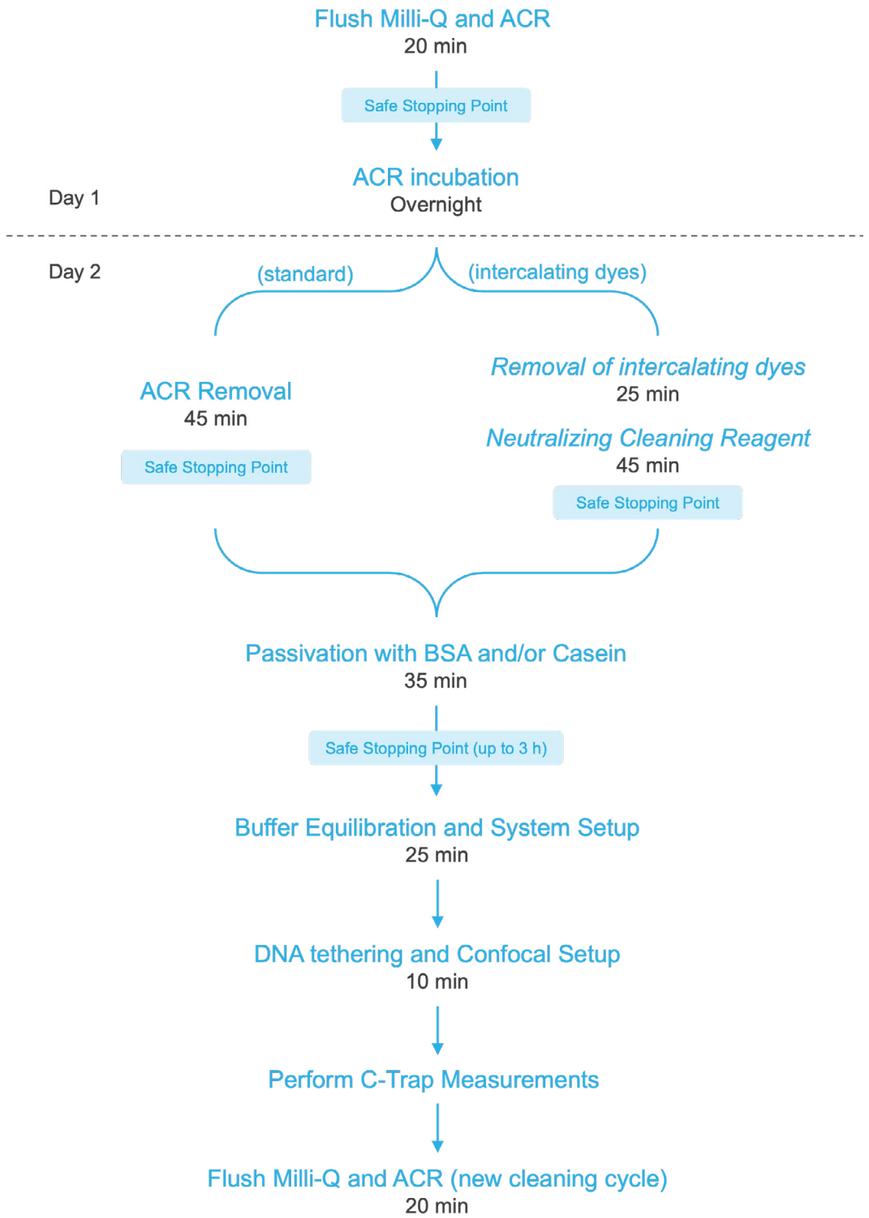
Recommended Materials

- Bovine Serum Albumin (BSA) – Sigma-Aldrich (Cat. No. A7906); dissolve in Phosphate-Buffered Saline (PBS)
- Thermo Scientific™ Blocker™ Casein – (Cat. No. 37582); ready-to-use.

Tip: During the cleaning and equilibration of the microfluidics module, use a 10 mL serological pipette with a pipette controller to aspirate 10 mL of buffer (depending on the protocol section), and dispense ~2 mL into each syringe, since precise microliter accuracy is not required.

Important: Replace the 10 mL serological pipette after distributing ACR to the syringes of the microfluidic module, before proceeding with the washing and equilibration steps.

3. Protocol Workflow



4. Cleaning and Passivation Protocol

Important: This procedure does not effectively remove DNA Intercalating Dyes. If such dyes (e.g., YOYO-1 or SYTOX Orange) have been used, refer to section 5 for the dedicated cleaning protocol with the Cleaning Reagent and Reducing Reagent.

4.1 Overnight Incubation with Alkaline Cleaning Reagent (20 min + overnight)

1. Fill all syringes with 1 mL Milli-Q water and flush all channels for 10 min at 2 bar.
2. Prepare 10 mL of 1x Alkaline Cleaning reagent (1x ACR) by diluting the stock ACR solution 50 times.
3. Remove the remaining water (~0.5 mL) from the syringes, add 2 mL 1x ACR to each syringe, and flush all channels for 30 min at 2 bar.
4. Incubate overnight (no longer than 72 h, see below step 4.2.4 for the long-term storage recommendation). Continue with Section 5.1 if removal of DNA Intercalating Dyes is required; otherwise, proceed with Section 4.2.

Tip: Before leaving the system for overnight incubation, confirm that ACR is flowing uniformly in all channels by visually inspecting that the ACR level is decreasing evenly in all channels. If the ACR level is decreasing more slowly in one channel (i.e., it seems blocked), try to resolve the block by unmounting the syringe from the pressure box and manually pushing ACR through that channel using a plunger. This should resolve the blockage and ensure uniform flow in all channels.

Important: The ACR incubation step should not be longer than 60 hours (e.g., it should not extend over the weekend). Longer ACR incubation times can negatively impact experimental results.

4.2 Alkaline Cleaning Reagent Removal (45 min)

Microfluidics Storage: After removing ACR and replacing it with Milli-Q water in step 7 of this section, the flow cell can be stored in Milli-Q water for up to one week. For longer storage, we recommend using Milli-Q water supplemented with Sodium Azide (0.1 mg/mL).

1. Remove the remaining 1x Alkaline Cleaning reagent from the syringes (~500 µL).
2. Replace the syringes on all channels with new syringes. Avoid splashing residual ACR onto the new syringe walls during the replacement.
3. To wash, add 2 mL Milli-Q water to all syringes, flush all channels for 10 min at 2 bar and remove the remaining water (~0.5 mL).
4. Repeat the washing step (step 4.2.3) two more times. After the last wash, do not remove the remaining Milli-Q water (~0.5 mL).

For long-term storage (>72 h) use a 0.1 mg/mL Sodium Azide solution after completing step 4.2.4.

Optional. Residual ACR can negatively impact experimental results. To maximize ACR removal, you can perform 3-5 rapid PBS washes before beginning passivation:

- Add 2 mL of PBS to all syringes and flush for 3 min at 2 bar.
- Remove the remaining 1.6-1.8 mL PBS from the syringes.
- Repeat this washing step 2-4 times.

4.3 Passivation with BSA or Casein (35 min)

Note: if passivation is not required, skip section 4.3 and continue directly with section 4.4.

In preliminary C-Trap protein binding tests, assess the optimal passivating agent (BSA, Casein, Pluronics, Tween etc.) for your assay. Below is our suggested protocol when using either BSA or Casein to passivate both channel 4 and 5.

Tip: To save time at the start of each experiment, prepare a 5x BSA solution (100 mg/mL) in PBS, make 200 µL aliquots and store at -20 ˚ C.

1. Remove the Milli-Q water from the syringes of channel 4 and 5 (protein channels).
2. Add 0.5 mL of 2% BSA in PBS or 1% Casein in PBS into the syringes of channel 4 and 5. Keep Milli-Q water in the remaining channels (top up to at least >0.5 m Milli-Q per syringe).
3. Flush all channels for 2 min at 2 bar, followed by 10 min at 0.5 bar (total flush volume ~200 µL).
4. Stop the flow and incubate for a minimum of 20 min (or up to a maximum of 3 hours).

Note: If the experiment cannot start immediately after passivation, the incubation in BSA and Casein can be extended to a maximum of 3 hours.

4.4 Buffer Equilibration (25 min)

To reduce the time between passivation and measurements, we recommend starting the instrument and optics setup in Bluelake during the buffer equilibration step. Detailed instructions on positioning the objective, condenser and microstage are available in the C-Trap User Manual.

1. Prepare 15 mL of buffer of choice.
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 10 min at 0.5 bar, and then remove the remaining buffer.
4. Repeat step 4.4.3 two more times. After the last repeat, remove the remaining buffer and proceed with adding your samples.

Note: Do not leave the Microfluidics Module in equilibration buffer for an extended period, as this may reduce the effectiveness of passivation.

5. Cleaning Protocol for DNA Intercalating Dyes (e.g. SYTOX, YOYO)

When intercalating dyes (e.g., YOYO-1 or SYTOX Orange) are used, an extended cleaning protocol involving Alkaline Cleaning Reagent (ACR), Cleaning Reagent (CR), and Reducing Agent must be applied.

The first part of the procedure (flushing with Milli-Q and ACR followed by overnight incubation) is identical to steps 1–4 in section 4.1.

5.1 Removal of DNA Intercalating Dyes with Cleaning Reagent (25 min)

Note: The Cleaning Reagent (CR) is not the same as the Alkaline Cleaning Reagent (ACR). The use of CR is required to effectively remove DNA Intercalating Dyes from the flow cell.

1. After overnight incubation of step 4.1, remove the remaining 1x ACR from the syringes (~0.5 mL).
2. Fill each syringe with 1 mL of Milli-Q water, flush all channels for 10 min at 2 bar and then remove the remaining Milli-Q (0.5 mL).
3. Fill each syringe with one mL of CR directly from stock (no dilution) and flush all channels at 2 bar for 10 min.

5.2 Neutralizing Cleaning Reagent with Reducing Agent (45 min)

1. Remove the remaining CR (~0.5 mL) from the syringes.
2. Replace the syringes on all channels with new syringes. Avoid splashing residual CR onto the new syringe walls during the replacement.
3. Prepare 10 mL of 1x Reducing Agent (1x RA) by diluting the stock RA solution 30 times.
4. Fill each syringe with 2 mL of 1x RA and flush all channels at 2 bar for 30 min.
5. Remove the remaining RA (≤0.5 mL) from the syringes.
6. Optional wash: add 1 mL of Milli-Q to all syringes, flush all channels for 10 min at 2 bar and then remove the remaining Milli-Q.
7. If passivation is required, proceed with section 4.3 and 4.4. If passivation is not required, add 1 mL of buffer to all syringes, flush all channels for 10 min at 0.5 bar, remove the remaining buffer and proceed with adding your samples.

For long-term storage (>72 h) use a 0.1 mg/mL Sodium Azide solution after completing step 5.2.5.

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