

# C-Trap Cleaning and Passivation Protocol

| Version 2.0

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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 ( $\varnothing$ )	Volume	Concentration (w/v)	Dilution factor
Streptavidin	1.0–1.4 $\mu\text{m}$	60 $\mu\text{l}$	1%	1:1000
	1.5–1.9 $\mu\text{m}$	60 $\mu\text{l}$	1%	1:1000
	4.0–4.9 $\mu\text{m}$	600 $\mu\text{l}$	0.5%	1:100
Anti-Digoxigenin	0.7–0.9 $\mu\text{m}$	600 $\mu\text{l}$	0.1%	1:100
	2.0–2.4 $\mu\text{m}$	600 $\mu\text{l}$	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.

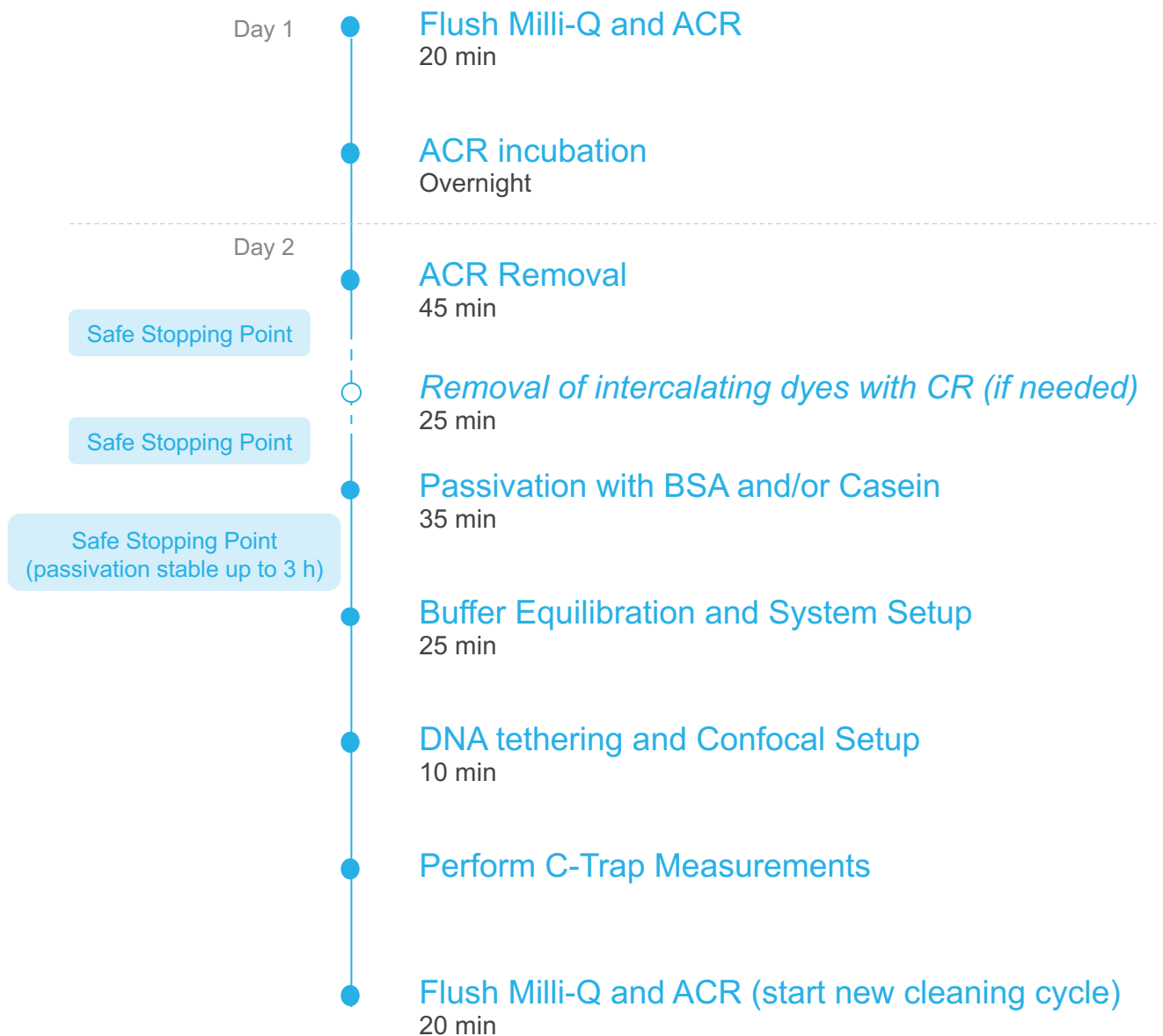


**Tip:** During the cleaning and equilibration of the microfluidics module, use a 10 ml serological pipette with a pipette controller to aspirate 10 ml of buffer (depending on the protocol section), and dispense ~2 ml into each syringe, since precise microliter accuracy is not required.



**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

1. Fill all syringes with 2 ml of Milli-Q water and flush all channels for 10 minutes at 2 bar.
2. Prepare 10 ml of 1x Alkaline Cleaning reagent (ACR) by diluting 200 µl of the 50x ACR solution with Milli-Q water.
3. Remove the remaining water from the syringes (~1.5 ml) and add 2 ml of 1x ACR to each syringe. Flush all channels for 30 minutes at 2 bar.
4. Stop the flow and incubate overnight.

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



**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 Alkaline Cleaning Reagent (ACR) from the syringes (~500 µl).
2. Replace the syringes on all channels with new syringes.
3. To wash, add 2 ml of Milli-Q water to all syringes. Flush all channels for 10 minutes at 2 bar.
4. Stop the flow and remove the remaining water (~1.5 ml).
5. Repeat steps 3–4 two more times. After the final flush, skip step 4 and keep the Milli-Q water in the system.

**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 minutes 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 and Casein

1. Prepare 1 ml BSA solution in PBS at a final concentration of 20 mg/ml to use immediately in the next step. Blocker™ Casein (1%) in PBS is provided as a ready-to-use.



**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.

2. Remove the Milli-Q water from the syringe of the protein channel.
3. Add 1 ml of freshly prepared BSA to channel 4 and Casein to channel 5. Leave 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 µl of BSA and Casein solutions have flushed through the protein channel.
6. Stop the flow and incubate for 20 minutes.



**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 and Optics Setup

To reduce the time between passivation and LacI binding 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 30 ml of your C-Trap assay buffer of choice.
2. Remove the remaining BSA and Casein solutions from the syringes.
3. Add 2 ml of assay buffer to each syringe. Flush all channels for 5 minutes at 0.5 bar. Stop the flow and remove the remaining buffer.
4. Repeat the washing step (step 3) two more times.
5. After the last wash, do not remove the remaining buffer.
6. Proceed immediately with DNA tethering and protein binding imaging.



**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

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.



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

### 5.1 Removal of DNA Intercalating Dyes with Cleaning Reagent

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.

### 5.2 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.

Proceed with passivation as described in section 4.3

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