Cleaning and Passivation - Legacy Protocol

Version 2.0

> This protocol has been discontinued. We highly recommend the cleaning and passivation procedures with the ***Alkaline Cleaning Reagent***, which rely on the materials provided by the ***Beads & Cleaning Kit – Recommended***.

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Cleaning and Passivation – Legacy Protocol

Materials supplied in the kit

Components	Units	Volume	Storage temperature
Cleaning reagent*	1	300 ml	Room temperature
Reducing agent	1	15 ml	Room temperature
Passivation buffer BSA 1%	4	4 ml	-20 ˚C
Passivation buffer Pluronic	1	15 ml	Room temperature

*The Cleaning Reagent (sodium hypochlorite, <5%) is different from the **Alkaline Cleaning Reagent (ACR)**.

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	Concentraion (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

Cleaning protocol

- Make sure all the valves of the flow cell are closed and that no pressure is applied.
- Remove any residual fluids from the syringes.
- Fill each syringe with 2 ml of Milli-Q water.
- Flush the flow cell with at least 1 ml of Milli-Q water by setting the pressure to 1.8 bar.
- Make sure all five inlet syringes (out of six total syringes) are flowing properly and that the flow rate is similar for all syringes. Slower flows in syringes may indicate partial clogging in the tubing. In this case, vent, close all valve except the one of the clogged syringe and the outlet. Disconnect the tubing from the flow cell and manually force with a syringe plunger water through the tubing. If it is still clogged, cut the very edge of the tubing as this is where proteins tend to accumulate. Repeat until it unclogs.
- Remove the remaining water from all syringes.
- Fill each syringe with 0.7 ml of cleaning reagent.
- Flush 0.5 ml of cleaning reagent into the flow cell, at a pressure up to 1.8 bar.
- Remove the remaining cleaning reagent from the syringes. Residual cleaning reagent that has remained on the syringe walls during this step, can be removed by using 1 mL of Milli-Q water to rinse the syringe by pipetting carefully up and down and take the water out before proceeding to the next step.
- Fill each syringe with 2 ml of Milli-Q water.
- Flush 0.5 ml of Milli-Q water into the flow cell with a pressure of 1.8 bar. In some cases, incorporating an extra step helps to clean remainders. In that case, right after flushing in the Milli-Q water, flush 0.5 ml 1 M HCl for 10 minutes with a pressure of 1.8 bar and wash the remains again with Milli-Q water before proceeding to the next step.
- Add 50 µl of reducing reagent (contains sodium thiosulfate) to the remaining 1.5 ml of Milli-Q water in each syringe to neutralize the remaining cleaning reagent. The volume of reducing reagent can be doubled in case cleaning reagent persists in the flow cell.
- Flush 1 ml of the diluted reducing reagent into the flow cell with a pressure of 1.8 bar.
- Remove the remaining diluted reducing reagent from the syringes.
- Fill each syringe with 2 ml of Milli-Q water.
- Flush at least 1 ml of Milli-Q water into the flow cell.

The flow cell is now clean for the introduction of your buffers, samples or any passivation protocol.

Passivation protocol

For DNA-binding protein experiments with the C-Trap, typically channel 4 and/or channel 5 are passivated. Below the passivation of Channel 4 is described.

- Use 450 µl of MiliQ to dilute 50 µl of passivation reagent BSA 10 times (obtaining 0.1 % BSA solution)
- Load in the syringe of channel 4.
- Expose the syringe, tubing, and flow cell for at least 20 minutes to the first passivation reagent by flushing it at a pressure of 0.3–0.4 bar.
- Use 450 µl of MiliQ to dilute 50 µl of passivation reagent Pluronic 10 times (obtaining 0.5% Pluronic solution). Remove the remaining 0.1% BSA solution from the syringe of channel 4 and add 500 µl of the 0.5% Pluronic solution.
- Expose the syringe, tubing, and flow cell for at least 20 minutes to the second passivation reagent by flushing it at pressure of 0.3–0.4 bar.
- Remove the remaining 0.5% Pluronic solution from the the syringe of channel 4, add your working concentration of protein buffer, and flow approximately 300 µl.

Your flow cell is now ready for introduction of your protein. Tip: better passivation can be achieved by overnight passivation of the protein solution before the experiment.

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