Cleaning and passivation protocol

Cleaning + BSA/Pluronics passivation

Consumables
Protocol
2022

LUMICKS

u-Flux flow cell cleaning protocol

When using any of the standard reusable LUMICKS flow cells, it is important to follow robust cleaning protocols between experiments to avoid cross-contamination. The protocol introduced here is based on the use of a cleaning reagent (contains bleach), which is able to irreversibly denature or destroy biological matter such as nucleic acids, proteins or even synthetic chromophores.

Materials supplied in the kit

Components	Units	Volume	Storage temperature
Running buffer 10x*	3	30 ml	Room 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%	1	15 ml	Room temperature

* The composition of running buffer 10x is PBS (1.37 M NaCl, 0.027 M KCl, 0.0119 M phosphates), 50 mM sodium azide and 5 mM EDTA. The pH is ~7.4.

Cleaning protocol

Important!

- Make sure to always pipette the solutions (cleaning reagent in particular) from bottom to top to avoid residues remaining in subsequent steps.
- Make sure there is never more than 2 ml of fluid in the syringe.

- 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 agent (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 agent can be doubled in case cleaning reagent persists in the flow cell.
- Flush 1 ml of the diluted reducing agent into the flow cell with a pressure of 1.8 bar.
- Remove the remaining diluted reducing agent 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.

The cleaning reagent contains bleach (sodium hypochlorite, <5%).

Store upright in a cool safe place. Keep bottle upright at all times.

***WARNING:** Causes skin irritation. Causes serious eye irritation. Harmful to aquatic life with long lasting effects. Keep out of reach of children. IF ON SKIN: Wash with plenty of water and soap. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If skin irritation occurs: Get medical advice/ attention. If eye irritation persists: Get medical advice/attention. Dispose of contents/container to normal waste streams. Avoid release to the environment. Warning! Do not use together with other products. May release dangerous gases (chlorine).*

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.

info@lumicks.com
www.lumicks.com

Or find us on:

LUMICKS HQ

Paalbergweg 3
1105 AG Amsterdam, The Netherlands
+31 (0)20 220 0817

LUMICKS USA

800 South Street, Suite 100,
Waltham, MA, 02453, USA
+1 781 366 0380

LUMICKS Asia

Room 545, Block A, Langentbldg Center
No.20 East Middle 3rd Ring Road
Chaoyang District, Beijing, 100022 China
+86 (0) 10 5878 3028

LUMICKS