Cleaning and passivation protocol (recommended)

Alkaline cleaning + casein passivation

When using any of the standard reusable LUMICKS flow cells, it is important to follow a robust cleaning protocol between experiments to avoid cross-contamination. After cleaning, the fluidics can be treated with a passivation buffer to prevent non-specific interactions of your proteins with the fluidics walls.

It is recommended that the cleaning protocol is run as soon as possible after an experiment is completed. This will keep the flow cell clean for a next experiment where passivation can be started immediately.

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LUMICKS

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Cleaning and passivation protocol

1. Cleaning and passivation materials supplied in the kit

Components	Units	Total volume	Storage temperature
Alkaline cleaning reagent	1	12 ml	Room temperature
Casein (1% solution)	1	3 ml	-20 °C
Cleaning reagent	1	300 ml	Room temperature
Reducing reagent	1	15 ml	Room temperature
10x Running buffer*	3	30 ml	Room temperature

* The composition of 10x running buffer is PBS (1.37 M NaCl, 0.027 M KCl, 0.0119 M phosphates), 15 mM sodium azide and 5 mM EDTA. The pH is ~7.4.

2. Cleaning protocol

Important!

- The new alkaline cleaning reagent is very effective at removing proteins, lipids, and beads. However, intercalating dyes like YOYO-1 and SYTOX Orange can be more difficult to remove with this reagent. If you're using these dyes, there are some additional steps added at the end of the regular cleaning protocol to ensure these dyes are destroyed.
- To avoid contamination of the alkaline cleaning reagent, make aliquots (e.g. 1 ml) and store at room temperature.
- Make sure to always fill syringes from the bottom to prevent any drops of reagents above the fluid meniscus.
- Make sure there is never more than 2 ml of fluid in the syringe.
- Regular cleaning using the cleaning liquid prevents bubbles from sticking in the flow cell.

If you are **using the new alkaline cleaning reagent (ACR) for the first time**, it's possible that there is significant residual buildup in the tubing, syringes and flow cell. This means that you will possibly see a lot of particles or debris flow by during and after the first cleaning cycle. To limit the effect on your workflows, we suggest following these steps for first-time use:

1. **Replace the syringes and flush 2 ml Milli-Q water.** *Syringes area large source of the residual buildup and should be replaced before use of ACR.*
2. Run the regular protocol and **ACR in the system overnight** after step 7.
3. **Flush with an additional 2 ml Milli-Q water after the regular protocol.** Continue flushing water until no more particles are seen.

With regular use of ACR, there should be less residual buildup in the system and therefore less need for additional flushing.

Regular protocol

When a regular cleaning cycle has been performed or experimental conditions have been altered significantly, it is advisable to replace all syringes. Any residues left on the syringes can potentially impact future experiments conducted under different conditions

1. Ensure all the valves are closed and no pressure is applied.
2. Remove any residual fluid from the syringes.
3. Add 1 ml of Mili-Q per syringe.
4. Flush for 2 min at 2 bar.
5. Remove any residual fluid from the syringes.
6. Dilute 0.2 ml of alkaline cleaning reagent (ACR) with 9.8 ml of Milli-Q water (50x dilution) and load 2 ml of the dilution to the syringes corresponding to the five channels of the flow cell.
7. Flush for 20 minutes with a pressure of 2 bar, or at least 1 ml. *Optional: The flow cell can be left overnight with ACR in case a thorough cleaning is needed, such as upon first-time use of the new ACR. Do not leave the flow cell with ACR for longer periods (>3 days). Typically, the flow cell should be stored in Milli-Q between experiments.*

Ensure that the flow rate is uniform for all syringes by checking that liquid levels have decreased evenly between syringes after flushing through sample/liquid. Uneven flow-rates can be caused by uneven inlet-tubing lengths or by partial clogging in the tubing. For troubleshooting the latter, see the troubleshooting notes at the end of the protocol.

8. Remove the remaining ACR from all syringes.

9. Rinse the syringe walls once with 1 ml Milli-Q (add and remove, no need to flush in this step).

10. (In case intercalating dyes have been used, using the regular cleaning cycle):

    a. Fill each syringe with 0.7 ml of cleaning reagent (sodium hypochlorite, <5%) (CR).

    b. Flush 0.5 ml of CR into the flow cell, at a pressure of 2 bar.

    c. Remove the remaining CR from the syringes. Residual CR 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.

    d. Fill each syringe with 2 ml of Milli-Q water.

    e. Flush ~10 minutes at 2 bar, or until about 0.5 ml water has flowed through

    f. Add 50 µl of reducing reagent (contains sodium thiosulfate) (RA) to the remaining 1.5 ml of Milli-Q water in each syringe to neutralize the remaining CR. The volume of RA can be doubled if CR persists in the flow cell.

    g. Flush 1 ml of the diluted RA into the flow cell with a pressure of 2 bar.

    h. Remove the remaining diluted RA from the syringes.

11. Add 2 ml of Milli-Q per syringe.

12. Flush for 20 minutes at 2 bar, then 1 min at 0.4 bar, or at least 1 ml.

The system is now ready for passivation.

Troubleshooting in case of partial clogging of the channels

- Vent and close all valves except the one corresponding to the clogged channel and the outlet. Disconnect the syringe from the flow cell and manually force water through the tubing with a syringe plunger.

- If the tubing is still clogged, cut the very edge of the tubing as this is where beads and proteins tend to accumulate. Repeat until it unclogs.

3. Passivation protocol

Important notes

- The 1% solution of casein provided with the kit should not show significant yellowing.
- To avoid thawing and freezing cycles of the casein, make 50 - 100 µl aliquots of casein (1% solution provided with the kit) and store them at -20 °C.
- Keep aliquots frozen and away from light as much as possible to prevent aggregates from appearing in the casein.

This protocol describes the passivation of a single channel.

1. Dilute casein to 0.05% in 1x PBS (25 µl casein 1% + 475 µl PBS).
2. Load 500 µl 0.05% casein solution per syringe of the channel to be passivated and 1.5 ml of your measurement buffer per syringe of each other channel.
3. Apply a flow of 2 bar for 2 minutes, then change the pressure to 0.4 bar and flow for an additional 10 minutes. Flush all channels (not just the passivated ones) to ensure proper laminar flow. Do not let the channels run dry.
4. Stop the flow and let the casein incubate for an additional 20 min without flow (you can lower this time at your own risk, though be mindful that this might only work for proteins that are less prone to sticking).
5. Remove the remaining casein solution from the syringe.
6. Add an additional 1 ml of your experimental buffer or buffer of choice to the syringes of the channels you have just passivated. Apply a flow of 2 bar for 2 minutes, then 0.4 bar for 10 minutes. Do not let the channels run dry.

Your flow cell is now ready for introduction of your protein samples.

4. Concise protocols

Alkaline cleaning reagent (ACR)

Dilute ACR (stock): 1mL stock + 49mL water and store at RT (up to 4 weeks).

Materials

Materials	Per channel	Per 5 channels (total)
ACR (dilute)	2 ml	10 ml
Water (MilliQ)	3 ml	20 ml

Steps

Notes (for first time use)

This reagent may remove buildup from the syringes and tubing that were not removed by cleaning reagent. To minimize the amount of buildup going through the flowcell, we suggest replacing the syringes before starting this protocol, leaving the ACR for a longer time (overnight), and flushing more water after the cleaning reagent step.

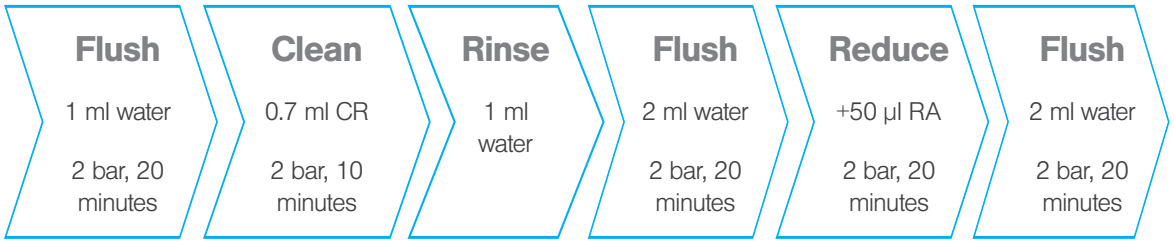
If intercalating dyes (SYTOX Orange, YOYO-1, etc) were used in this experiment, we recommend also using Cleaning reagent after this procedure.

Cleaning reagent (contains sodium hypochlorite, <5%)

Materials

Materials	Per channel	Per 5 channels (total)
Cleaning reagent (CR)	0.7 ml CR	3.5 ml CR
Reducing agent (RA)	50 µl RA	250 µl RA
Water (MilliQ)	4 ml	30 ml

Steps



Notes

Protect cleaning reagent (CR) from light. Do not store dilute reducing reagent (RA) for > 1 day.

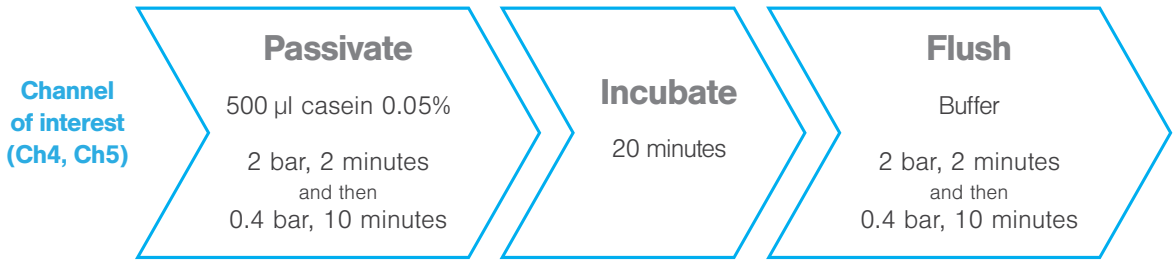
Passivation

Passivate channels of interest for components of low concentrations (< 5 nM) or known to be highly interactive or stick to surfaces (proteins).

Materials

Materials	Per channel
Casein	25 µl casein 1% 475 µl PBS
Experiment buffer	1 ml

Steps



Other channels: same series of steps, but with *Experiment buffer only*. (2 ml per channel).

Notes

We recommend making aliquots (50 µl) of the casein to avoid excessive freeze-thaw cycles.

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