LSA and LSA^{XT} Maintenance & Best Practices Guide

Required Maintenance

After Every Surface Prep Array

- Prime the PH into H_2O + 0.05% T-20.

After Every Capture Kinetics Experiment

- Prime the PH into H_2O + 0.05% T-20.
- Use A DI H_2O-soaked TechniCloth (or lint-free) wipe to gently ***blot*** and wipe the underside/gasket of the ***SFC*** to remove any salt build-up. Capture Kinetics first drips buffer into the chip bath using the printhead, then docks the SFC into the buffer pool; as buffer evaporates, crystalline salts can form and compromise the SFC to chip seal.

Weekly

- Check LSA/LSA^{XT} PC for Windows Updates
  - If not using the recommended Bridge PC setup, install updates and enable the Pause Windows Updates feature.
- Restart LSA/LSA^{XT} PC
  - Backup experiment data (copy Documents/Carterra/Experiment Data to external storage).
- Run a System Prime
  - Ensure the cleaning slides are cleaned with DI H_2O and 70% IPA/EtOH before docking on slides.
  - Prime SFC and PH on the cleaning slides (H_2O + 0.05% T-20).
- Clean PH Face
  - A DI H_2O soaked TechniCloth (or lint-free) wipe can be used to gently blot the tip of the PH that interfaces with the cleaning slide.
- Run a System Clean
  - In Navigator, under "Cleaning and Maintenance," select Clean.

As Needed or After Supernatants

- Clean Face of SFC
  - A DI H_2O soaked TechniCloth (or lint-free) wipe can be used to gently blot and wipe the underside/gasket of the SFC to remove any buildup.
- Prime Based Cleaning Procedure (to flush the full B1/B2 tubing length up to the bulkhead)
  - 3 X System Primes on the Cleaning Slides with 0.1% Bleach, 3 X System Primes on the Cleaning Slides with 3% Contrad, 3 X System Primes on the Cleaning Slides with DI H_2O + 0.05% T-20

Quarterly

- Run a System Sanitize
  - Navigator –> Cleaning and Maintenance –> Sanitize.
- Before running experiments, run 3 system primes with H_2O + 0.05% T-20.
  - Prime the SFC and PH on the cleaning slides, ensuring they are cleaned between each prime.

Before Extended Idle TimeRun a Sanitize +Sleep

- In Navigator, under "Cleaning and Maintenance," select Sanitize + Sleep. After the Sanitize, you will be prompted to move the lines to an empty bottle.
  - Close Navigator and leave the LSA/LSA^{XT} and computer powered ON.

After Extended Idle Time

- Install any pending Windows updates if not using the Bridge PC setup and Reboot the LSA/LSA^{XT} computer.
- Place all the lines (B1, B2, H_2O, C1, C2) in a bottle of H_2O + 0.05% T-20 and run a Sanitize under Cleaning and Maintenance.
  - Ensure the cleaning slides are cleaned with DI H_2O and 70% IPA/EtOH before docking on slides.

Best Practices

Before Every Prime

- Clean the glass slides with DI H_2O and 70% IPA/EtOH before docking on slides.

For Every Experiment

- Run **two primes** to ensure the buffer is fully exchanged
- After the experiment, prime into H_2O + 0.05% T-20.

Chip Preconditioning (Perform as the first experiment on a new chip)

- Universal chip preconditioning protocol: [4] x 1 min. injections of 20 mM NaOH followed by [2] x 1 min. injections of 10 mM NaOAC pH 4.5.
- Ensure the raw, unprocessed data is being charted between ~5K-10K RU for the optimal detection range (Nav. 2.1 and higher automatically apply reference subtraction on the Experiment Runner. This should be unapplied to view raw data).

Buffer Preparation

- Ensure the buffer is fully homogenized by inverting the running buffer bottle at least 8 times to mix.
- Filter and degas the homogenized running buffer.
- Set some of the buffer aside for Sample Preparation.
- Ensure line B2 is not placed into BSA containing running buffer to avoid multichannel clogging.

Sample Preparation

- EDC/S-NHS
  - Mix the reagents together as close to the start of the experiment as possible.
  - Prepare the final concentrations at 50-75 mM EDC and 25 mM S-NHS for single channel lawn activations. Prepare the final concentration at 15 mM EDC/5 mM S-NHS for multichannel side activations
- Quench
  - Use Ethylenediamine every time, to quench the activation and reduce the chip charge (see 1x Bulky Buffer).
- Buffer
  - Prepare fresh daily to avoid contamination.
  - Keep running buffer at room temperature.
  - Invert the bottle at least 8 times to ensure the solution is fully homogenized
  - Degass the running buffer.
- Ligand
  - Ensure the concentration allows ≥10 fold dilution into 10 mM sodium acetate for direct coupling (final salt < 20 mM to avoid ionic competition).
  - When possible, immobilize at different densities and in replicates.
  - Supernatants should be diluted at minimum 2-fold.
- Analyte
  - Buffer-exchange the proteins into running buffer to avoid refractive-index mismatches.
  - Ensure the tubes and plates do not have bubbles at the bottom of the wells.

Thermals

- Allow the Interaction temperature to equilibrate to the set temperature with the SPR chip inserted for at least 45 min prior to starting the experiment or performing chip normalization.

Chip Reuse

- Do not use a capture lawn more than 15 times.
- When storing lawns for later use, fill the chip bath with ~2 mL of 25-50% glycerol 50-75% running buffer in an airtight Tupperware container at 4˚C.
  - Ensure the glycerol solution is removed from the surface before reusing. Reusing capture lawns after storage significantly reduces the usability/quality of the surface.
- When re-inserting a lawn in the LSA/LSA^{XT} ensure the glass prism sides are clean and free of spots. If present, clean with a 70% IPA/EtOH solution.

Experimental Method

- Kinetics
  - Ensure the method includes enough leading buffer blanks to achieve a stable/flat injection profile to use as the leading blank when analyzing.
  - Keep kinetics R-max ≤150 RU for generally lower ligand densities that result in more accurate measurements.
  - Long Capture Kinetics (multiple sets > ~8 hours): Queue each set as a separate Capture Kinetics experiment to ensure proper wetting (this occurs only during the first print of the first set). Merge the resulting files into one .kitx to analyze all sets together.
- Epitope
  - Immobilize ligand mAbs to at least 200 RU for robust signals.
  - Identify proper Regeneration conditions that achieve complete regeneration of the Ligands.
  - When binning > 192 Abs, break the experiment into two separate experiments.
  - Ensure two buffer or Ag controls are included at the start of each binning experiment.

Analysis Software

- Ensure you are using the latest released version of the Analysis software. This can always be found on our website: https://carterra-bio.com/resource-category/software

Consumables

- Ensure only Carterra approved and validated plasticware is used: https://carterra-bio.com/store

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