

Standard Operating Procedure (First draft)

Control software login:

User: cytiva
Password: biacore

To maintain the system's performance, sanitization protocols will be run once a month or earlier when dimmed necessary. System check protocols will be run monthly, and results will be available.

All buffers and large-volume reagents should be filtered through a 0.22 μ m filter to avoid introducing unwanted particles into the flow system. Particles can lead to SPR response disturbances and cause blockage or other malfunctions in the microfluidic system.

Do not use polystyrene microplates with samples that contain DMSO.

Aliquots of stock of common reagents will be in the fridge (ask for the key)
Materials (provided by a nominal \$30 fee)

Desorb solutions (1,2).

Desorb 1: 0.5% w/v sodium dodecyl sulfate, **Desorb 2:** 50mM glycine-NaOH pH 9.5. (Desorb kit: Cytiva BR-1008-23 ~ 250 ml solutions) **Flow buffer:** 10 mM HEPES pH 7.4, 150 mM NaCl, 3.4 mM EDTA, 0.005 % v/v P20. All solutions at temperature > 20 C
Desorb1 and Desorb2 are provided

P Surfactant (to add to user's buffers):

500 μ l aliquots of 10% P20 will be provided to be dissolved in a liter of the buffer (target concentration 0.005% v/v).

Flow Buffer:

10 X HEPES + P (10X HBS-P) and 10X PBS +P in 50 ml aliquots

Microplates, foil, and septas

96 wells plates (preferable round bottom, BR199577) with the cytiva® seals

96 deep wells block

Additional Materials and cost

A maintenance chip will be in the machine.

Bionormalizer solution aliquots

Sensors chip CM5 (\$ 250).

Buffers: 10X HBS+P PBS+P, P20, "Bionormalization" solution

Getting Started

- 1) Book your time in **Ilab** (which will include the booking procedure). Notify the manager of your intention to use the Biacore (ideally, two days before) independently to have booked the time.
- 2) Log your starting time and user info (**Name, time, method used, buffer used**) in the logbook provided. The software also logs methods and results in the Biacore database for the user's benefit.
- 3) Log in to the system if it is not already.
- 4) You will find the Biacore in standby mode, with a maintenance chip loaded, **and it must be left in the same state after use**. If the system is in standby mode (the instrument status panel at the bottom of the screen shows *Running standby flow*), no further action is needed. Standby mode will be stopped automatically when a new instrument activity is started.
- 5) Optionally, desorb protocol (running time 14 min) before starting your experiments and between different samples. It will improve the quality and reproducibility of your data.

Be sure that a maintenance chip is in the machine. Fill deep-wells block A1-8 wells with 880 μ L of Desorb solution 1 and wells B1-8 with 880 μ L of Desorb solution 2 and place the block in the hotel position A.

15 ml of each solution (enough for two desorb operations) will be provided. Place the block in position A and run the desorb protocol on the control page.

- 6) Unload the maintenance chip, save it in the bag provided, place it in the fridge, and load the appropriate chip for your run.
 - a) Add a **Change chip** to the activity queue.
If a sensor/maintenance chip is already docked in the instrument, click **Undock chip** and
 - b) remove the chip from the sensor chip port.
 - c) Select **New chip** and enter the details of the sensor chip for the run. Alternatively, select **Existing chip** and choose a chip from the list.
 - d) Insert the sensor chip with arrows pointing to it and visible, and close the chip door.
 - e) Click **Dock chip (2 min)**
 - f) *For runs requiring the highest performance, allow the flow system to equilibrate in standby mode overnight after changing the sensor chip or solutions. Extend the equilibration time to at least 24 h if the detergent concentration in the running buffer changes.*
 - g) Run the **change buffers using buffer and reagents protocol**

- 7) Inside the BIACORE software. Make a directory (use PI name/your name) to store your protocols and results, whether they still need to be added.
- 8) Set up your method and run your experiments.

To pause between runs.

Place water and reagent inlets in the water and the buffer inlet in your buffer. Then, run the standby protocol.

Exit Protocol

- 1) Remove your chip () and load the maintenance chip (as described in step 6 of Getting started)
- 2) **Desorb again** (as 4 of Getting started).
- 3) **Clean-up after the run.** The following activities should be performed as required after a run:
 - i. Remove any plates from the sample hotel. Clean the station.
 - ii. Remove the reagent bottle and replace the reagent pipe bottle with a **filtered Milli-Q water bottle (2L)**.
 - iii. Empty the waste bottle.
 - iv. Run **standby** (bottom left) in the activity queue. **Ensure there is 2L** of or sufficient liquid for the intended standby period (2L last 3 ½ days).
 - v. If necessary, clean the drip tray.
- 4) Log your exit and e-mail mbianch1@jhmi.edu (please use BIACORE in the subject).