Octet RED^{96} Start up/Shut down Protocol Simplified

STOP This protocol is for qualified users operating Octet RED^{96} in IBC 402 only. Dr. Jao accepts no responsibility for actions taken as a result of using this protocol. Reading the manufacturer's software user guide is highly recommended.

Set up DummyEXP -> sensor hydration -> Labeling reaction -> Spin column -> Ligand loading -> Kinetic method -> Analyte series dilution

Start up procedure:

1. Turn on control PC. (The instrument is always left on.)
2. Double-click Octet data acquisition software.
3. The instrument initializes automatically. It may make a loud noise due to its failure to locate right-most position.
4. Choose **Instrument: Reset** to establish connection. The auto-sampler checks positions for a warm-up.
5. Choose **Experiment: Set Plate Temperature** to 30℃.
6. Biosensors should be soaked in hydration buffer for at least 15 minutes in sensor tray for conditioning and equilibration prior to usage. The hydration buffer is usually your experiment buffer except for some sensors (e.g. APS sensors) which might be water.
7. BCF provides NHS-LC-LC-biotin labeling reagent, which is about 0.3μL 10mM aliquot in 100%DMSO for preparing biotinylated proteins. To one aliquot of labelling reagent, add 60μL 50μM of your protein for a one-to-one molar ratio reaction. (example: Use 20μL 150μM BSA per tube, i.e. 20μL 10mg/mL BSA).
8. At the end of the biotinylation reaction, use desalting columns (or spin columns) to get rid of unreacted labeling reagent. Depending on the labeling efficiency, approximate 100 - 500nM of ligand is needed to load SA (SAX) sensors.
9. Please make sure the green rack and plates are seated correctly. If the blue heater can be seen, the position of black plate is wrong!!
10. Before start your big experiments, perform a non-specific binding test by using a blank sensor with your sample to check if there is binding of your sample to the sensor surface. If non-specific binding does occur, the buffer needs optimization. Adding 0.05% Tween20, 0.1mg/mL BSA or PEG400 may help. Otherwise, use different type of sensors. Please note that the following sensors may contain streptavidin: SA, SAX, AHC, AMC, Fab2G, NTA, His1K, HIS2, GST and CHO. If needed, ask for 1ug/mL biocytin to block unoccupied biotin binding sites on the

sensor surface. It is advised to design your experiments with double reference subtraction to eliminate background that is caused by buffer mismatch or insignificant non-specific binding.

11. Buffers, reagents and samples (180 -220 μL) are filled in a 96-well plate, which is placed on the temperature controlled orbital shaker sample stage.
12. Close the instrument door. The door may be open only when the instrument status window shows “ready”.
13. Start a basic kinetics or quantitation experiment by wizard or a previously saved method (.fmf). ForteBIO templates can be found in “C:\Data\TemplateFiles”. Sinica methods can be found in “C:\Data\SinicaMethods”.
14. Follow the set-up procedure tabs from left to right, i.e. defining the sample plate, defining an assay, assigning biosensors to samples, reviewing and running experiment.
15. The default directory of experimental data is C:\Data
16. Please refer to Octet Data Analysis Software 10.0 User Guide for data analysis.

Shut down procedure:

17. Take the sensor tray and plate out.
18. Turn off control PC and monitor.
19. Leave Octet RED^{96} instrument on.