mAb affinity measurement using the Octet® system based on Bio-Layer Interferometry (BLI) technique

A. Introduction

Bio-Layer Interferometry (BLI) is one recently developed technique and applied to Octet® systems by FortéBIO. BLI is a layer of molecules attached to the tip of an optic fiber which creates an interference pattern at the detector, any change in the number of molecules bound causes a measured shift in the pattern. Octet systems enable real-time, label-free analysis for determination of affinity, kinetics and concentration from biomolecular interactions in 96- or 384-well microplates. Here, we will apply Octet QK system on the affinity measurement of the specific antigen-monoclonal antibody (mAb) binding.

B. Materials

1. The Octet QK system:

Data Acquisition and Data Analysis software shall be installed.

Each experiment requires 2 black flat bottom polypropylene 96 well microplates (Greiner Part Number 655209). Plate 1: Pre-wetting plate for biosensors Plate 2: Plate for buffers, reagents and samples

Dip and Read Biosensors: Anti-Mouse IgG Fc Capture.

2. Reagent and Buffer:
Antigen and mAb, 1xPBS (phosphate-buffered saline)

C. Methods

1. Turn on computer first.

2. Turn on instrument (toggle switch on separate power supply). (**See note 1**)

3. Start software: Data Acquisition 7.1 and set up Kinetics Assays.

3.1. Use experiment templates for optimization assays, here we choose: Experiment-Templates-Kinetics-biomolecule kinetics AMC biosensors

3.2. Enter samples information in the plate definition. The concentration is in MOLAR terms and at least 1 well of buffer (0nM Sample) for referencing is included. Here we use 1xPBS as buffer, load list is mAbs at concentration 20ug/mL, and samples list is antigen solutions at 3 different concentrations: 20ug/mL, 10ug/mL, and 5ug/mL, the molecular weight (KDa) for antigen is needed. The volume of buffer, mAb and antigen is 200uL. (**See note 2**)

3.3. Assay definition: Equilibration in buffer (180s), loading mAb solution (600s), baseline (180s) in buffer, association (900s) in antigen solution, dissociation (1800s) in buffer. The shaking speed is 1000 rpm. (**See note 3**)

3.4. Sensor assignment: we choose 7 AMC biosensors, the first 3 biosensors are for one pair of antigen-mAb, and the second 3 biosensors are for another pair of antigen-mAb. The last AMC biosensor is for reference. (**See note 4**)

3.5. For more details, please check the Data Acquisition User Guide provided by OCTET QK system.

4. Assign samples to 96 wells and put it in the OCTET QK system, set temperature at 25°C and

start the experiment. (**See note 5**)

5. Start software: Data Analysis 7.1.

5.1. Select the data file to Loaded Data, double click to open it.

5.2. Change the 'well type' for the zero concentration of sample from 'sample well' to 'reference well' and then select Subtract reference well. Align Y axis to baseline, change time range between 5 seconds, then process data.

5.3. Open 'Analysis' tab and choose association and dissociation options. Choose 1:1 model for antigen-mAb reaction and fit curves.

5.4. Save report. K_{on}, K_{off} and K_D are in the report for different concentrations of antigen.

5.5. For more details, please check the Data Analysis User Guide provided by OCTET QK system.

D. Notes

1. The instrument should be on for at least 1 hour prior to running an experiment.

2. Remember to put into the concentration of mAb and antigen.

3. The analysis software needs Baseline, Association and Dissociation steps consecutively to work.

- If the K_D is in the nM range, an association of 10 minutes and a dissociation of 10-20 minutes may be sufficient to obtain kinetic constants with low error.
- If the K_D is < 1 nM, an association of 15 minutes and a dissociation of 30-60 minutes

may be necessary to obtain kinetic constants with low error.

- If the K_D is unknown, err on the long side for both association and dissociation, skip ahead in acquisition software, and/or shorten data analysis times in the software.

4. The biosensors require a minimum of 10 min of pre-wetting in the Pre-wetting plate prior to running experiment.

5. Make certain the biosensor tray and sample plate are fully seated in the instrument to avoid crashing the biosensors. The system will finish the experiment by itself. Leave instrument on unless it will be idle for an extended period of time. Turning on and off every day will greatly shorten the lamp life. The lamp should last between 1-3 years of regular use.