Bio-Layer Interferometry (BLI) - Octet RED96

In a nutshell: High-throughput, kinetic binding analysis using the 96-well plate format and eight parallel detectors. Ready-to-use biosensors for antibodies, as well as for biotinylated, His- and GST-tagged proteins.

Services: Biophysics Facility offers Octet as an open-access instrument. First-time users must complete a short training session before gaining access to the instrument reservation calendar. Training includes a full analysis of a standard protein-protein interaction.

Location: Building 50, room 3226

Description: Octet RED96 employs BLI (Bio-Layer Interferometry, a technique similar to SPR) to simultaneously collect data from up to eight “dip-and-read” sensors. This instrument uses 96-well plates for sample handling. The molecule of interest ("ligand") is immobilized on the surface of a sensor, creating the biolayer. When this sensor is dipped into a well containing a solution of the ligand’s binding partner ("analyte"), the analyte molecules bind to the immobilized ligand molecules, changing the biolayer’s thickness. To monitor the biolayer thickness changes, Octet analyzes the interference of the light beam reflected from the tip of the sensor. In this way, Octet can track the association and dissociation of the analyte from the immobilized ligand in real time.

BLI sensors are user-customizable, and several types of premade sensors are available, including Streptavidin, NTA, A/G/L- Protein, and amine-reactive surfaces. Most sensors can be regenerated and used multiple times. Octet RED96 can use crude sample solutions, such as cell culture supernatants, diluted serum, etc. The BLI signal is sensitive to the binding of a large range of analytes, from small molecules (MW > 150 Da) to virus particles.

Typical applications:

- measurements of binding kinetics and affinity
- off-rate ranking
- epitope binning
- antibody and protein concentration measurements
- automated, quantitative ELISA assays

Basic instrument specifications:

- Temperature: room temperature or temperature stabilized in the 28°C to 40°C range
- k_a: 10^2-10^7 $M^{-1}s^{-1}$
- k_d: 10^{-6} – 10^{-1} s^{-1}
- K_D: 10 pM – 1 mM
- Small molecule sensitivity limit ~150 Da
- Large molecule sensitivity limit ~200 nm
- Sample format: 96-well microplates, 180-200 µL sample volume, no sample consumption or transfer, samples are fully recoverable
- Number of simultaneous detection channels (sensors): 8
- Maximum data acquisition rate: 10 Hz
- Time required for a single analysis (eight samples, including controls and the dilution series): Approximately 30 to 45 minutes, depending on the kinetic rates of binding. Additional time is required to setup the microplates.

Sample requirements and recommended buffers:

PBS buffer with the addition of 0.02% Tween-20 to prevent the non-specific interactions is recommended, but Octet can handle a wide range of buffer compositions. Addition of up to 0.1% BSA or Casein is also used to combat the non-specific interactions. Glycerol should be avoided if possible, but if necessary, it must be kept at concentration of no more than 2% v/v, and the concentrations must be carefully matched between the buffer and analyte solutions.

It is also necessary to carefully match the buffers in the analyte solution and in the well that will be used to establish the pre-binding signal baseline. This is best done by dialysis or a similar procedure.

Minimum sample amount: A minimum of 400 µL of both the ligand and analyte solutions is required to set up the plate with the necessary controls (a binding-check experiment).

NTA sensors require His-tagged molecules at a concentration of 20 µg/mL or higher.

SA sensors require biotinylated molecules in the 5 to 25 µg/mL concentration range.

The analyte solution (solution of molecules interacting with the immobilized ligands) should be available at a concentration of at least 10x its dissociation constant value; if the dissociation constant is unknown, we recommend a concentration of 1 µM or higher.

The standard biotinylation protocol requires either 100 µL of a 2 mg/mL protein solution, or 500 µL of a 0.4 mg/mL protein solution. Biotinylation protocols that require smaller amounts of protein exist, but are more difficult to control and may not yield the desired results.

Consumables:

We stock several types of BLI sensors, including SA (streptavidin) for biotinylated ligands, NTA and Anti-Penta-His for His-tagged molecules, Anti-Human IgG Fc, and others. Consumables are provided at the manufacturer's prices