Octet red96e

Bio-layer interferometry (BLI)

Bio-layer interferometry is a method that measures biomolecular interactions on a surface in real time. One interacting partner is immobilized on the surface of sensor tip while the second one is in solution. The binding is detected via fiber optics as a change in interference pattern of light reflecting from the sensor surface. Similarly to surface plasmon resonance, it enables to determine the binding kinetics, affinities and also perform the quantification experiments. The main advantages of BLI technique are virtually no sample consumption (measurement directly in plates), high speed and possibility to perform the measurement also in highly heterogeneous environment (such as blood plasma).

BLI can be used for:

- testing of protein activity
- specificity determination – searching for binding partners, characterization of inhibitors affinity, tests for cross-reactivity
- affinity (kinetics) – kinetic and equilibrium parameters of an interaction, the rates of complex formation (k_a), dissociation (k_d), and equilibrium association/dissociation constants can be determined
- direct test of expression of a given protein in cell line cultures
- concentration determination – concentration is determined by monitoring the interaction of a molecule with a prepared sensor surface after the callibration by set of analyte of known concentrations
- multiple interaction during complex formation – complex formation can be monitored as each component is incorporated into a multimolecular complex

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Technical specifications

Instrument: Octet RED96e (Forte Bio)

Features:

- High-quality kinetic screening and affinity characterization
- Up to 8 assays in parallel
- Detects a diverse range of biomolecules from peptides to proteins to mammalian cells
- Accessible range of affinity constant (K_D) 1 mM – 10 pM, association rate constants (k_a) from 10^1 to 10^7 M^{-1} S^{-1} and dissociation rate constants (k_d) from 10^{-6} to 10^{-1} s^{-1}
- Quantitation range 0.05 µg/mL to 2000 µg/mL
- Analysis temperature range: ambient + 4 (typically 25) up to 40°C
- Up to 96 samples can be analyzed in a single run
- An optional microplate evaporation cover minimizes sample loss enabling up to 12 hours of unattended run time
- Fluidics-free format reduces assay time and maintenance cost

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Instrument operation

Assemblies:

- Sensors available: streptavidine-coated, anti-His-tag, amin reactive. Broad range of other sensor types is available by the producer, can be ordered upon special request.

Data evaluation SW:

Operational mode:

BLI measurement is performed by the users themselves after training.

Provided services:

- Instrument user training
- Basic BLI data evaluation training
- Consulting/assistance

Sample requirements

- Sample volume is 180–220 µL/well, sample can be fully recovered
- Almost all buffers are compatible with the technique, various components such as glycerol, DMSO, bME, etc. can be used
- Immobilization of one interacting partner is essential. Choose wisely the sensor that will be used for immobilization of your sample. If you are in doubt, ask for expert consulting on site to minimize the risk of sensor degradation.
- Technique sensitivity depends on the size of interacting partner. If possible, try to immobilize the smaller partner. For protein-protein and protein-nucleic acid interactions, choose based on sample availability and stabiity of individual molecules.
- Each sensor might be used multiple times, however a suitable regeneration procedure needs to be applied. If you do not know the stability of your immobilized partner, you might need to try regeneration scouting or test your system separately via other biophysical methods (e.g. circular dichroism, light scattering)

It is recommended to discuss the project and the details of the experiment (sample preparation, sample requirements) with the Core Facility members in advance.

Contacts

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Instrument Location:

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