Microfluidic Diffusion Sizing (MDS) - Fluidity One-M

In a nutshell: Measurements of interaction affinity, stoichiometry, ligand concentration, and molecular size in native conditions, including serum, plasma, and cell lysate.

Services: Biophysics Facility offers MDS as an open-access instrument. First-time users must complete a short training session before gaining access to the instrument reservation calendar. Training includes the K_D determination of a standard molecular interaction. During the MDS training, you will use our pipetting robot to quickly prepare the dilution series for titration.

Location: Building 50, room 3123

Description: In the MDS experiment small amounts of sample and buffer solutions are drawn through the microfluidic channel in two parallel laminal streams. This allows for the unconstrained diffusion of molecules from the sample to the buffer stream. The extent of sample diffusion will depend on the experimental parameters (buffer viscosity, temperature, etc.) and on the size (hydrodynamic radius, R_h) of the diffusing molecules. The rate of the diffusion is measured by the ratio of the fluorescence intensity of the labeled target molecules detected at the sample and buffer exit ports at the end of the microfluidic channel. Ligand binding to the target increases the hydrodynamic radius of the molecule and slows its diffusion rate. Consequently, a smaller amount of target molecules will diffuse to the buffer stream and a lower target fluorescence intensity will be detected in the buffer exit port. Assessing the extent of the sample diffusion provides information on the size of the molecular complexes and can be used to evaluate the target molecule state and quality, as well as the target-ligand binding. Unknown ligand concentration can be evaluated by global analysis of binding titrations performed at different concentrations of the target molecule. Labeling target molecules with fluorescent probes provides measurement specificity. This allows performing binding analysis in complex media such as serum, plasma, and cell lysate.

Typical applications:

- Measurements of binding affinity in protein-protein and protein-nucleic acid interactions
- Measurements of interaction stoichiometry
- Measurements of the active target concentration in complex media (plasma, serum)
- Measurements of molecular size (free and bound probe)

Basic instrument specifications:

- Fluorescence Sensitivity: 100 pM – 10 µM; recommended target concentration ≥ 5 nM
- Molecular mass range: R_h; 1 nm – 20 nm; M_W; 1.4 kDa – 14 MDa
- Media viscosity range: 0.82 cP – 1.73 cP (from pure water to 20% Glycerol)
- Measurement wavelengths: Green 488 nm or Red 647 nm
- Chip-plates: 24 sample capacity – 24 MDS circuits, 7 µL volume capacity each.

Sample requirements and recommended buffers: Target molecule has to be fluorescently labeled. GFP, YFP or FITC can be used for detection in the green channel, and Cy5 or Alexa647 for detection in the red channel. Red channel detection usually has a lower level of background fluorescence and for this reason should be used for MDS measurements in complex media. MDS experiments can be performed in a wide range of biologicals buffers and media.

Minimum sample amount: The MDS circuits have approximately 7 µL capacity and require 3.5 µL of both sample and buffer solution for loading. A single chip-plate has 24 circuits, and the typical binding titration includes three repetitions of 8-point serial dilutions and uses a whole plate. The labelled target concentration should be lower than the expected K_D (typically 5 nM – 50 nM). Approximately 0.24 mL of the target solution is required to prepare the 3x8 titration series.

The highest ligand concentration in the titration series should be ten times higher than the expected K_D. This will require approximately 10 µL of the 20xK_D ligand stock solution to prepare.

Larger target amounts will be required for labelling, depending on the labelling protocols. Additional target and ligand amounts may be required for tests and control experiments.

Consumables: MDS chip-plates have a 24-sample capacity, and any number of sample circuits can be used in a measurement. Plates are RFID coded to track the used circuits and allow the partially-filed pates to be conveniently used in another experiment.

Consumables are provided at the manufacturer's prices.