

## Mass Photometer (MP) – Refeyn OneMP

**In a nutshell:** Native state, label-free and immobilization free single-molecule mass analysis in solution.

**Services:** Biophysics Facility offers MP as an open-access instrument. First-time users must complete a short training session before gaining access to the instrument training calendar. Training includes mass distribution analysis of a standard protein sample.

**Location:** Building 50, room 3226

**Description:** MP is a single-molecule method that analyzes the molecular mass of particles in solution in their native conditions. It can be used to characterize proteins, DNA, RNA, and large molecular assemblies, including protein complexes, oligomers, ribosomes, viruses, and other biomolecules. A drop of the sample solution at an approximately 10 to 20 nM concentration is pipetted onto a glass coverslip. The MP instrument measures the contrast of the images of individual molecules in the sample solution. These images are created by the interference of the illumination light with light scattered by the particles. Importantly, the contrast of these images correlates with the particle molecular mass. Since the MP signal is light-scattering based, samples do not require labeling or immobilization. However, there is no specificity of the light-scattering based detection and impurities that will interfere with measurements must be removed from the buffer. MP measurements not only use a very small amount of material for analysis, but are also very fast. MP samples do not require any special preparation and the data collection and analysis takes only a few minutes.

### Typical applications:

- Determination of sample heterogeneity, purity, polydispersity
- Oligomerization analysis
- Measurements of biomolecular interactions ( $K_D$ )
- Analysis of proteins and protein complexes, DNA, RNA, and lipids

### Basic instrument specifications:

- Accessible mass range: 40 kDa – 5 Mda
- Accuracy of the mass determination:  $\pm 2\%$
- Component mass resolution: up to 19 kDa (in the low molecular mass range)
- Sample consumption: as low as 10 ng per measurement
- Sample concentration range: 1-100 nM
- Image size: from 10.8  $\mu\text{m}$  x 2.9  $\mu\text{m}$  (regular) to 10.8  $\mu\text{m}$  x 10.8  $\mu\text{m}$  (large)

- Illuminating laser power: approximately 400 mW
- Analysis time: < 5 min

**Sample requirements and recommended buffers:**

- Buffers used for MP experiments have to be carefully filtered using a syringe filter
- The preferred buffer is PBS, but this method can accommodate a wide range of buffer compositions. Avoid glycerol at concentrations higher than 2% and the low-salt buffers.
- Since MP requires no labeling, samples must be of a high purity: Impurities and any molecules with a molecular weight greater than 40 kDa will interfere with detection.

**Minimum sample amount:**

- Sample volume: 10  $\mu$ l (after the final dilution)
- The final sample concentration should be in the range of 5 to 50 nM, diluted from the stock of micromolar concentration just prior to the experiment.

**Consumables:** We stock pre-cleaned coverslips that can be used out-of-the-box. With the standard well-gasket, each can be used to prepare six sample wells. We also stock the aliquots of the unlabeled protein ladder and BSA for mass calibration.

Consumables are provided at the manufacturer's prices:

A charge for one coverslip will be added to all reservations. Please enter a note in iLab with the actual amount of coverslips used in the experiment.