

MassFluidix™ HC system for rapid dilution via microfluidics

Refeyn's MassFluidix High Concentration (HC) is a microfluidics system that extends the analytical strength of mass photometry to samples with high concentrations of proteins. The system makes it possible to characterize binding, oligomerization and protein-protein interactions for samples with concentrations up to the tens of micromolar.

The role of concentration in mass photometry

Mass photometry is a bioanalytic technology that measures the mass distribution of biomolecules in a sample in solution without the need for labels. For optimal mass photometry measurements, the concentration of the sample to be measured should typically be less than 100 nM. While this concentration is often ideal for studies in native conditions, in some cases there is a need to analyze samples at a higher concentration.

Enabling measurements at high concentrations

To expand the concentration range for mass photometry analysis, the MassFluidix HC system (Fig. 1) uses microfluidics technology to raise the upper sample concentration limit from the nanomolar to the micromolar range. It enables a measurement to be made at a concentration optimal for mass photometry, while capturing the state of a biomolecular system at micromolar concentration. MassFluidix HC is an add-on for Refeyn's TwoMP mass photometer, and includes a fluidic control box and rapid dilution chips (Fig. 2).



Fig. 1 Refeyn's MassFluidix HC system for rapid dilution via microfluidics. The picture shows a complete mass photometry setup, including MassFluidix HC (left, orange square), the TwoMP mass photometer on an Accurion anti-vibration unit (center) and a computer that controls the instruments (right). Compatible with Refeyn's TwoMP mass photometer, MassFluidix HC enables the analysis of high-concentration samples by mass photometry.

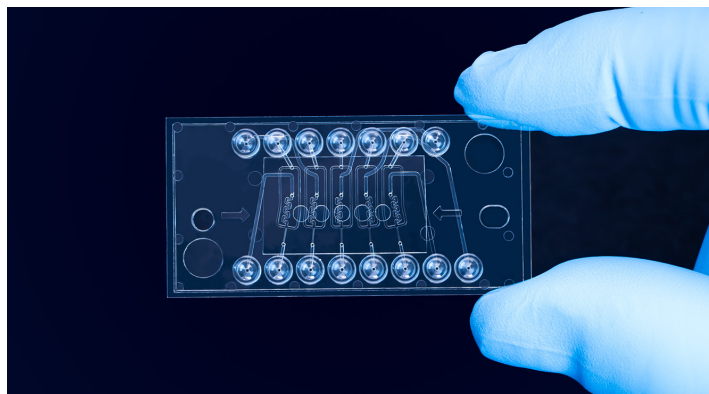


Fig. 2 Refeyn's MassFluidix HC chip for rapid dilution. Sample dilution and measurement take place in each of the chip's five channels.

How does MassFluidix HC work?

MassFluidix HC works by rapidly diluting the sample and flowing it across the measurement surface in milliseconds – before the biomolecular system's equilibrium has been disrupted by the dilution. It enables, for example, the detection and characterization of low-affinity complexes that are unstable at a low concentration (Fig. 3).

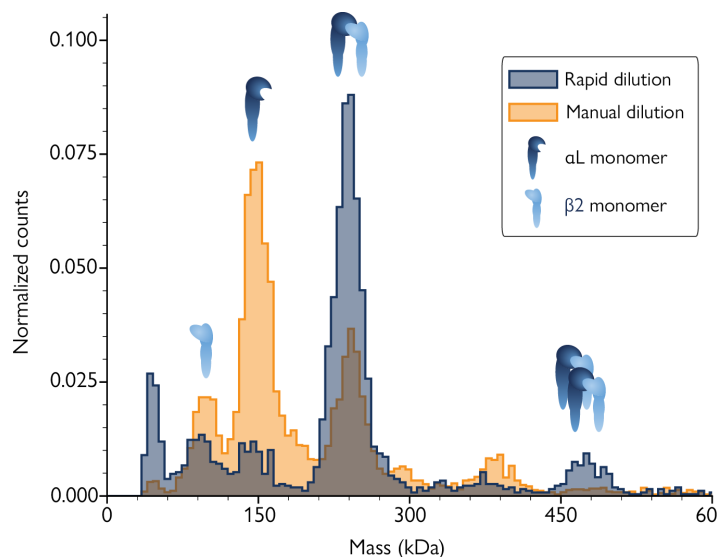


Fig. 3 Rapid dilution with MassFluidix HC reveals low-affinity complexes. A 2 μ M solution of integrin α L/ β 2 was measured with mass photometry after being diluted to 5 nM either manually or using the MassFluidix HC system. Following manual dilution (orange), α L and β 2 monomer peaks were clearly visible, along with a small heterodimer peak. In contrast, after rapid dilution with MassFluidix HC, there were fewer monomers and significantly more heterodimers present, in addition to heterotetramers – which were poorly resolved after manual dilution. Samples were obtained from R&D Systems Inc.

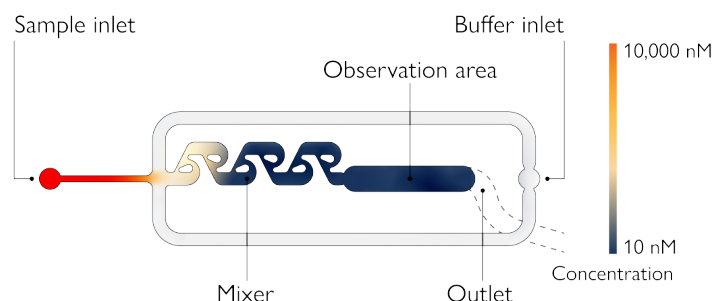


Fig. 4 The sample is rapidly diluted on the MassFluidix HC chip, as show in this illustration of one channel on the chip. The undiluted sample (orange) and the buffer (grey) enter through inlets. Sample dilution occurs where the channels meet and in the mixer, reaching the target concentration (dark blue) near the observation area. Fluid color indicates concentration (see colorbar). Each chip has five channels.

The MassFluidix HC chip for rapid dilution

Sample dilution and the mass photometry measurement occur on the MassFluidix HC chip. The dilution factor is set prior to the initiation of the experiment. Dilution takes place when the sample and buffer are combined in a reverse Tesla valve mixer, then the diluted sample flows through the observation area before leaving the chip through an outlet (Fig. 4). The mass photometry measurement is done in the observation area. Each chip (also available from Refeyn) has a set of five such dilution and measurement channels (Fig. 2).

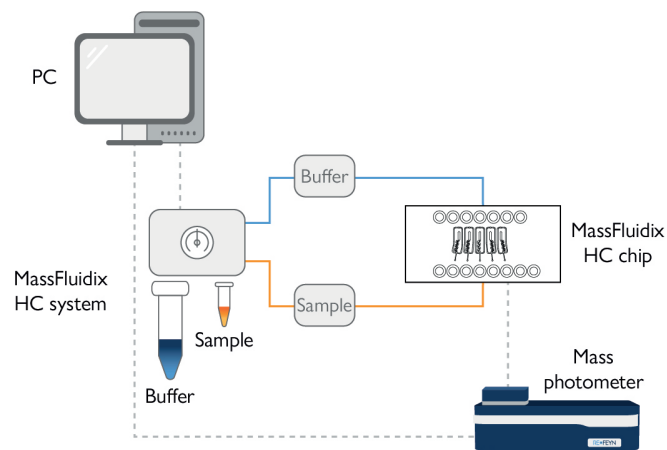


Fig. 5 Overview of the MassFluidix HC system. The computer (top left) is connected to the MassFluidix HC central unit (shown adjacent to the buffer and sample tubes) and the mass photometer (bottom right). Flow rates through the sample and buffer lines are tightly monitored. The sample and buffer line tubing connects to a channel on the MassFluidix HC chip, which is placed inside the mass photometer.

The MassFluidix HC system

The MassFluidix HC system (Fig. 5) consists of multiple components: A computer, a central unit, sample and buffer line tubing, and flow rate monitors. These components work together to ensure that the sample and buffer enter the chip at the correct flow rates. The whole system is automatically detected by Refeyn's software and controlled from AcquireMP. This careful control enables rapid sample dilution, which is immediately followed by the mass photometry measurement.

MassFluidix HC key specifications

MassFluidix HC system

Buffer flow rate	1 mL/min
Sample flow rate	0.1 – 8 μ L/min
Mass range	50 kDa – 5 MDa
Max sample dilution	10,000 X
Max starting conc.	50 μ M
Required sample vol.	~20 – 60 μ L, depending on flow rate
Measurement time	20 minutes
Cleaning time between samples	14 minutes
Sample dilution time	<37 ms (inlet to observation area)
Compatibility	TwoMP
Dimensions	180 x 315 x 280 mm (WxDxH)
Weight	~2 – 3 kg
Power outlets	2 additional required

Components supplied Pressure source, fluidic control box, power supply and MassFluidix HC Starter Kit (including tubing, connectors, top stage plate and modified lid)

MassFluidix HC chip

Number of channels	5 per chip
Shelf life	6 months
	Chips with unused channels can be reused for 10 days after opening
Storage	Room temperature

Note: The MassFluidix HC system also enables static mass photometry measurements.

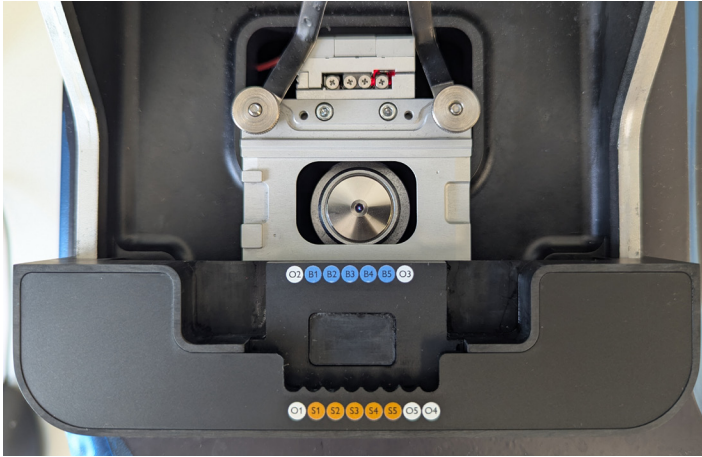
How to buy: Chips are available individually as part of MassFluidix HC Sample Preparation Kits (**MP-CON-21027**), which also contain 2 bags of mini leuc connectors and a box of lens cleaning tissue.

Related products: Also available are MassFluidix HC Sample Preparation Plates (**MP-CON-51014**), which contain a sample preparation plate compatible with the TwoMP with MassFluidix HC.

For more information, visit the [Refeyn website](https://refeyn.com) or send an inquiry to orders@refeyn.com.

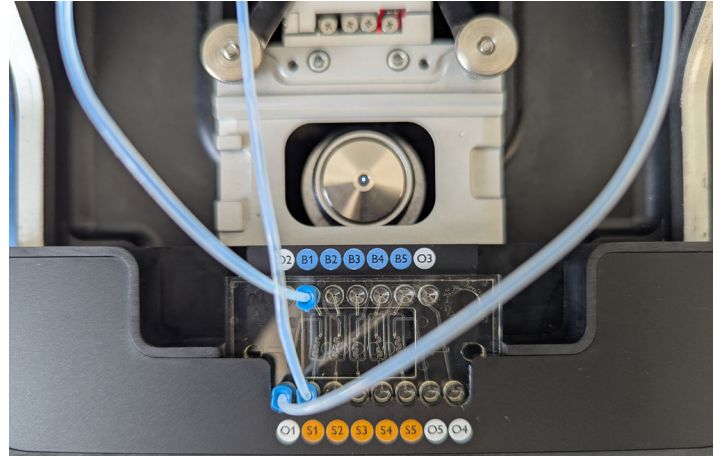
How to use the MassFluidix HC sample preparation plate

The sample preparation plate is a component used to aid the user in setting up the connections of the MassFluidix chip. It includes a slot to hold the chip securely and markings that indicate the sample inlet, buffer inlet and outlet for each channel.



Step 1: Place the preparation plate on the lid of the mass photometer stage.

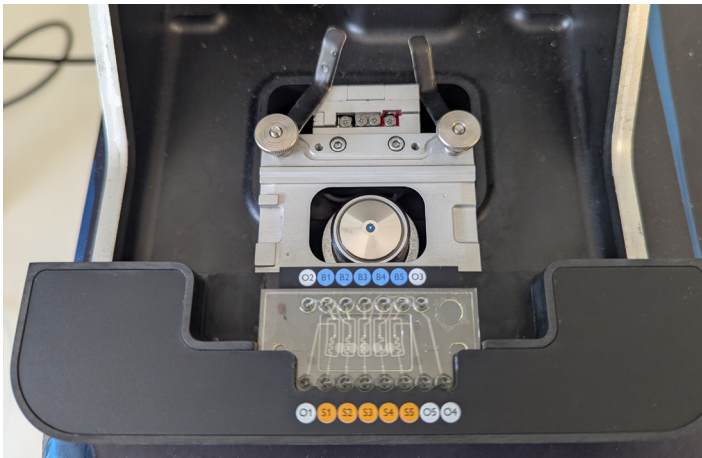
Mass photometers compatible with MassFluidix include a modified lid to allow the necessary tubing through. The sample preparation plate is designed to fit on these lids.



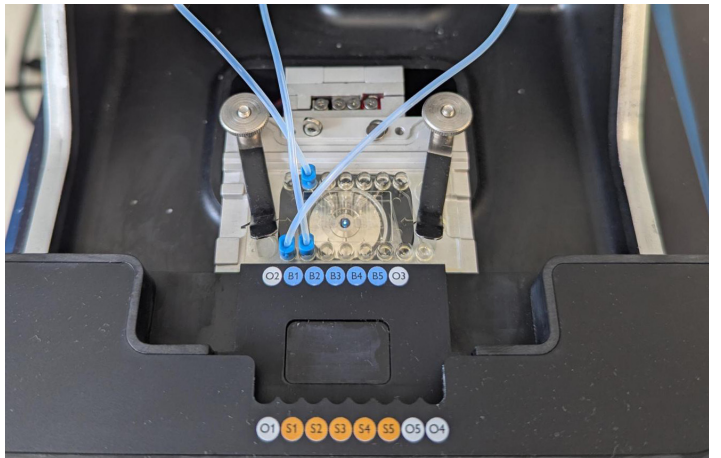
Step 3: Use the guides to connect the tubing

Choose which channel to be used for your measurements and make the appropriate attachments using the guide on the sample preparation plate.

In the example above channel 1 is being used, so the user will connect the buffer inlet to B1, the sample inlet to S1 and the outlet to O1.



Step 2: Slot the MassFluidix chip into the chip holder on the sample preparation plate.



Step 4: Place the chip on the stage and remove the sample preparation plate.