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Version 1

Mass photometry V.1

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Protocol status: Working

We use this protocol and it's working

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Abstract

A protocol for performing mass photometry measurements.

- 1 Clean the highe-precision coverslips (Azer Scientific) by alternating between isopropanol and water.
Repeat the above treatment for 3 times in a bath sonicator, each 3 min.
Air dry the coverslips
- 2 Clean the gasket by alternating between isopropanol and water.
Repeat the above treatment for 3 times, without sonicatino.
Air dry the gasket.
- 3 Add 19 ul of mass photometry buffer (30 mM HEPES pH 7.4, 5 mM MgSO₄ and 1 mM EGTA) to the well for autofocus
- 4 Dilute the protein sample in the mass photometry buffer to a concentration of 5–20 nM.
- 5 (Optional) For crosslinking, add 1 µL of 0.1% (v/v) glutaraldehyde in mass photometry buffer to 9uL protein sample and incubated for 10 minutes, followed by quenching the reaction with adding 10 µL of 1M Tris-HCl pH 7.4.
- 6 Prior to the measurement, perform mass calibration using a standard mix of conalbumin, aldolase, and thyroglobulin. Analyze the mass photometry profiles and fit them to multiple Gaussian peaks, with the mean, standard deviation, and percentages calculated using DiscoverMP software (Refeyn).
- 7 Measure the protein contrast counts using Refeyn TwoMP mass photometer, with three technical replicates.