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🌐 Size Exclusion Chromatography with Multiangle Light Scattering (SEC-MALS)

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Protocol status: Working

We use this protocol and it's working

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Abstract

SEC-MALS


Troubleshooting



- 1 Concentrate the purified protein sample to 4-5 mg/ml

- 2 Instruments:
Agilent 1200 HPLC system (Agilent Technologies, Santa Clara, CA)
Wyatt DAWN HELEOS-II MALS instrument
Wyatt Optilab rEX differential refractometer (Wyatt, Santa Barbara, CA)
WTC-050S5 size-exclusion column (Wyatt)
40 ul sample loop

- 3 Equilibrate the system and column with buffer containing:
25 mM HEPES pH 7.5, 200 mM NaCl, 1 mM MgCl₂, 2 mM TCEP

 Overnight

- 4 Turn on the laser 1 hour before the experiment.
Confirm the readout is stable

- 5 Run 5 mg/ml BSA to calibrate the system

- 6 Inject the sample and run (takes ~1 hour/sample)

- 7 Analyze the results by ASTRA V software (Wyatt).
MALS signals, combined with the protein concentration determined by refractive index,
are used to calculate the molecular mass of the complex.