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**Protocol status:** Working  
 We use this protocol and it's working

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

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ABSTRACT

SEC-MALS

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- 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 MgCl<sub>2</sub>, 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.