

# BIOMOLECULAR COMPLEX PURIFICATION: DYNAMIC LIGHT SCATTERING



## DYNAPRO NANOSTAR II DLS

DynaPro™ NanoStar™ II can measure hydrodynamic size ( $R_H$ ), size distributions, particle concentration and weight-average molar mass ( $M_w$ ) as well as the second virial coefficient (A2 or B22), the diffusion interaction parameter (kD), turbidity or opalescence and the temperature of protein melting ( $T_m$ ) or aggregation onset ( $T_{onset}$ ). Method is applicable to  $R_H$  range from 0.2 nm to 1000 nm, temperature from +25 °C to +120 °C (Biocomplex has no cooling option) and  $M_w$  from 300 Da to 1 MDa. Thus, it can be used for the analysis of versatile samples such as proteins, viruses and virus-like particles, liposomes, nanoparticles, and more. Data is collected and analyzed using Dynamics software.



DynaPro NanoStar II is a cuvette-based DLS instrument (Wyatt). It measures dynamic light scattering and static light scattering (90°). Laser wavelength is 662 nm.

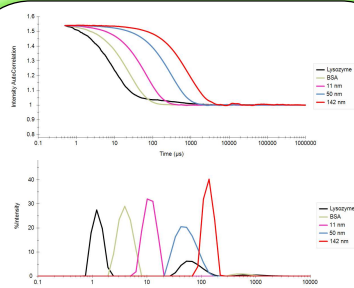
## Biocomplex has:

- Quartz cuvette: minimum sample volume 2  $\mu$ L
  - Size, particle concentration\* and  $M_w$ \*
  - +25 °C up to +120 °C
- Disposable cuvettes: minimum sample volume 4  $\mu$ L
  - Size
  - +25 °C up to +80 °C

\*Applicable, if radius is smaller than ~150 nm

## Standards:

- BSA:  $R_H$  3.8 nm
- Polystyrene beads:  $R_H$  10, 20, 30, 50, 60, 142 nm



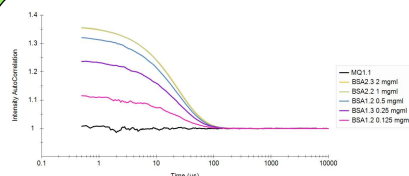
Autocorrelation function and size distribution of lysozyme ( $R_H$  ~2 nm), BSA ( $R_H$  ~3.8 nm) and standard polystyrene beads with  $R_H$  of 11, 50 and 144 nm. Data shows that the lysozyme sample contained aggregates.

**Biocomplex** provides facilities for ultracentrifugation, asymmetric field flow fractionation, batch mode DLS and chromatography. Our technologies can be used to analyze and purify large macromolecular complexes such as **nanoparticles, membrane vesicles, protein complexes, polymers** etc. from biological, synthetic and environmental samples.

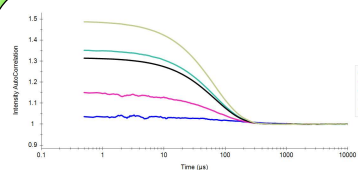
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## SAMPLE REQUIREMENT

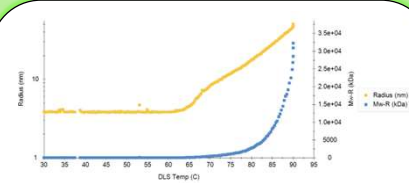


Two-fold dilutions of BSA to test the measurement sensitivity and reproducibility: autocorrelation functions. BSA stock concentration was 2 mg/ml. The Y-axis value should be above 1.1.



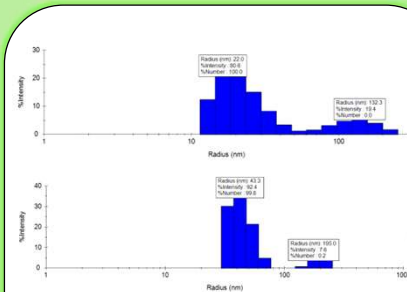
Ten-fold dilutions of standard beads with  $R_H$  of 11 nm (stock ~ $3 \times 10^{15}$  particles/ml): autocorrelation functions. **Note!** Sensitivity is dependent on the  $M_w$  that correlates with the light scattering intensity. Thus, less sample is needed for higher  $M_w$  samples. Particle concentration and  $M_w$  measurements require information on sample concentration and dn/dc.

## SAMPLE STABILITY

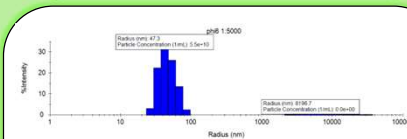


Temperature ramp test from 30 to 90°C: melting curves show the change in  $R_H$  and  $M_w$  of BSA as a function of increasing temperature. **Note!** Stability tests can also be performed at constant temperature or by increasing the temperature step wise.

## SAMPLE QUALITY



Size distribution (%intensity and %number) and presence of aggregates: purified phiX174 and phi6 viruses.



Size ( $R_H$ ), polydispersity (%PD), particle concentration: purified phi6 virus. Data shown is from one measurement with five 5 sec acquisitions.

Item	Sample	Radius (nm)	%PD	Intensity	Normaliz.int.	Baseline	Amplitude	SOS	Forward Monitor	Particle Concentration
1	Acq1	47.3	19.7	1310	8415	1.001	0.417	6.325	0.31	5.44e+10
2	Acq2	45.3	15.1	1027	6222	1.001	0.415	5.584	0.31	7.06e+10
3	Acq3	45.5	24.1	1394	8538	1.002	0.430	6.745	0.31	7.07e+10
4	Acq4	46.8	15.0	1325	8082	1.001	0.421	6.650	0.31	6.70e+10
5	Acq5	47.8	8.4	1317	9911	1.001	0.420	6.530	0.28	5.46e+10

## Biocomplex has also the following services:

- Ultracentrifugation: 6 ultracentrifuges and 8 rotor types (fixed angle and swing out, ThermoScientific / Sorval)
- BioComp Gradient master for gradient making
- BioComp Piston Gradient Fractionator with Triax flow cell: A260, A280, eGFP or Cy5.
- ÄktaPure25M chromatography instrument
- Asymmetrical flow field flow fractionation: Eclipse NEON (Wyatt) with MALS, DLS, dRI, UV and fluorescence detectors.
- Electric asymmetrical flow field flow fractionation