

HELSINGIN YLIOPISTO HELSINGFORS UNIVERSITET UNIVERSITY OF HELSINKI

BIO- JA YMPÄRISTÖTIETEELLINEN TIEDEKUNTA BIO- OCH MILJÖVETENSKAPLIGA FAKULTETEN FACULTY OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES

Hilife **BiocompleX**

BIOMOLECULAR COMPLEX PURIFICATION: ULTRACENTRIFUGATION & FRACTIONATION









ULTRACENTRIFUGATION

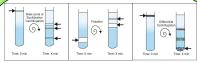
Biocomplex ultracentrifugation service provides facilities for rate-zonal, equilibrium, flotation and differential centrifugation. It is equipped with a farm of ultracentrifuges as well as swing out and fixed angle rotors with different capacities. Thus, also preparative purification are possible. Biocomplex also has instrumentation for gradient making and provide fractionation services accompanied with UV data collection.

ULTRACENTRIFUGATION FACILITY

- Six ultracentrifuges (Thermo Scientific Sorvall WX Ultra Series) +/- HEPA filters.
- Swing-out and fixed-angle rotors with different speed and volume capacities
 - TH641, AH629, AH650,
 - F50L, T1270, T865, T647.5, A621
- Gradient making instrument (BioComp Gradient master)
- Piston Gradient Fractionator with Triax three wavelength flow cell for UV (A260 and A280) and fluorescence (eGFP or Cy5) profiling (BioComp).
- Beckmann Airfuge for fast pelleting of small sample volumes



CENTRIFUGATION TECHNIQUES



Rate zonal and equilibrium centrifugation use density gradient solutions. In rate zonal centrifugation. particles move at different rates depending on their sedimentation coefficients. In equilibrium centrifugation, particles form zones at the position that is equivalent to their buoyant density. In flotation centrifugation sample components with density higher than that of the gradient sediment, and particles that are lighter float to the top. Separation in differential centrifugation is based on the size and pass of the particles.

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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PISTON FRACTIONATION

Biocomplex fractionation instrument utilizes the topdown method where the piston descends from the top, displacing layers through the center and keeping smooth flow. The method enables minimal disturbation of the gradient.

Fraction collection is combined to data collection on UV and eGFP or Cy5 traces. Piston tips are compatible with the following tubes:

- TH641 (SW41, 6*12 ml)
- AH650 (SW50.1 tubes, 6*5 ml)
- AH629 (6*36 ml tubes)

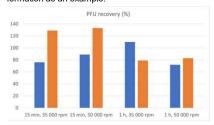


Fractionation of empty and genome-filled virus particles after ultracentrifugation in 5-20% sucrose gradient. PRD1 as an example.

APPLICATIONS



Analysis of the assembly of biomacromolecular complexes in sucrose gradient and their purification for further analysis. Phi6 polymerase complex formation as an example.



Fast recovery of virus samples using F50L rotor that has capacity for 24 1.5 ml tubes. Pelleting of phi6 virus is shown as an example. Initial virus concentrations were 2.5 *109 PFU/ml (blue) or 3*10¹⁰ PFU/ml (orange).

Biocomplex has also the following services:

- Ultracentrifugation: 6 ultracentrifuges and 8 rotor types (fixed angle and swing out, ThermoScientific /
- BioComp Gradient master for gradient making
- BioComp Piston Gradient Fractionator with Triax flow cell: A260, A280, eGFP or Cy5.
- ÄktaPure25M chromatography instrument
- DynaPro NanostarII (Wyatt) batch mode dynamic light scattering instrument