

Shared Instrumentation Core in the Biomolecular Research Facility

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<http://www.healthsystem.virginia.edu/internet/biomolec/home.cfm>

The Shared Instrumentation core provides access to several user operated instruments and chromatography, biomolecular interactions and gel scanning services. All instruments are in Jordan Hall, in the Biomolecular Research Facility.

User fees cover a portion of the costs of maintaining the instruments and services, and funds from the School of Medicine support the core.

User Operated Instruments

Fluorescent plate reader

Molecular Devices Spectra MAX Gemini EM. A computer running Spectramax software controls the instrument and acquires data, which can be exported to an Excel compatible text file.

This instrument is maintained under a service contract

- Fluorescence and some luminescence readings on 6, 24, 96, 384 well plates.
- Kinetic readings.
- Dual monochromator instrument for wide range of fluorophores

- Accessible all hours with appropriate access card permissions
- Some common assays are Alamar Blue and PicoGreen for quantitating DNA.



Fluorescent plate reader located in Jordan Hall room 1074

Circular Dichroism Spectropolarimeter

Aviv Instruments model 410

- CD spectra, which can be used to estimate protein secondary structure.
- Circular dichroism at specific wavelengths to observe unfolding with temperature.
- Monitor changes in structure
- Accessible all hours with appropriate access card permissions

Aviv software controls the instrument and can analyze data. Raw data can also be exported to an Excel compatible text file.



Aviv 10 CD located in Jordan Hall room 1074

Dynamic Light Scattering Protein Solutions Dyna Pro

This instrument provides estimates of the size of proteins in solution.

2-D Electrophoresis Equipment

- Amersham IPGphor isoelectric focusing apparatus with holders for 18 and 25 cm strips
- Bio-Rad Protean isoelectric focusing apparatus with trays for 11, 17 and 25 cm strips
- Bio-Rad Criterion Dodeca electrophoresis apparatus for running 12 Bio-Rad Criterion gels
- Bio-Rad Protean Xii cell for running 2 cm gels with 17 cm strips
- Bio-Rad Dodeca Plus electrophoresis apparatus for running up to 12 gels, 17 to 25 cm wide.
- Bio-Rad Rotofor for isoelectric focusing in solution

Core Operated Services

Chromatography by reverse phase, ion exchange and gel filtration.

For reverse phase separations, sample size can be from sub microgram to 50 milligrams.

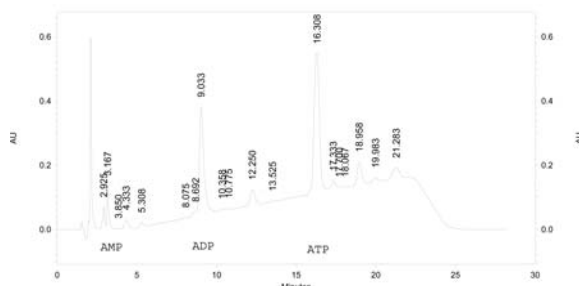
Equipment:

- FPLC (Pharmacia/Amersham), for ion exchange and gel filtration.
- Dionex Ultimate 3000 for capillary/microbore. This system can monitor at 2 wavelengths.
- Beckman HPLC. These systems are for larger samples run on 4.6 to 22 mm diameter reverse phase columns.

The core has some columns but investigators may need to purchase columns for some projects.

Applications:

- Separation of vaccine peptides from Human Immune Therapy Center and collection for analysis by the Mass Spectrometry Core.
- Analysis of nucleotides.
- Isolation of chelating compound from microorganisms
- Quantitation of some drugs.



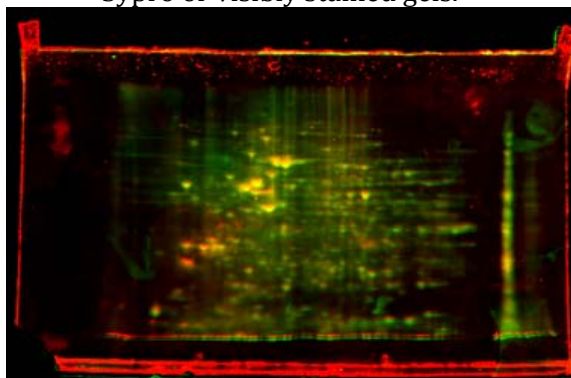
Nucleotide separation by ion exchange chromatography



Dionex U-3000 for small scale chromatography

Gel Scanning and spot picking

- Bio-Rad FX to scan gels stained with Sypro Ruby, ProQ phosphoprotein stain, CyDyes, and other fluorescent dyes.
- Bio-Rad GS-800 densitometer for transmissive scanning of gels stained with Coomassie or silver.
- The scanners produce images which can be quantitated and converted to TIFF files.
- Bio-Rad Spot Cutter cuts cores from Sypro or visibly stained gels.



HEK cells: overlay of Sypro stain (green) and phosphoprotein stain (red)

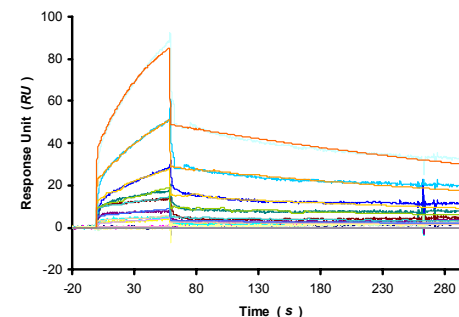
Biomolecular Interactions by Surface Plasmon Resonance

This service uses a Biacore 3000 instrument for estimation of association and dissociation rates and constants.

One compound is bound to a coated gold surface by covalent coupling, or antibody binding. Surface plasmon resonance measures the amount of an analyte bound to the ligand on the surface as a function of time.



Biacore 3000 instrument



Binding of the proteinase jararhagin to collagen 14 coupled to a chip