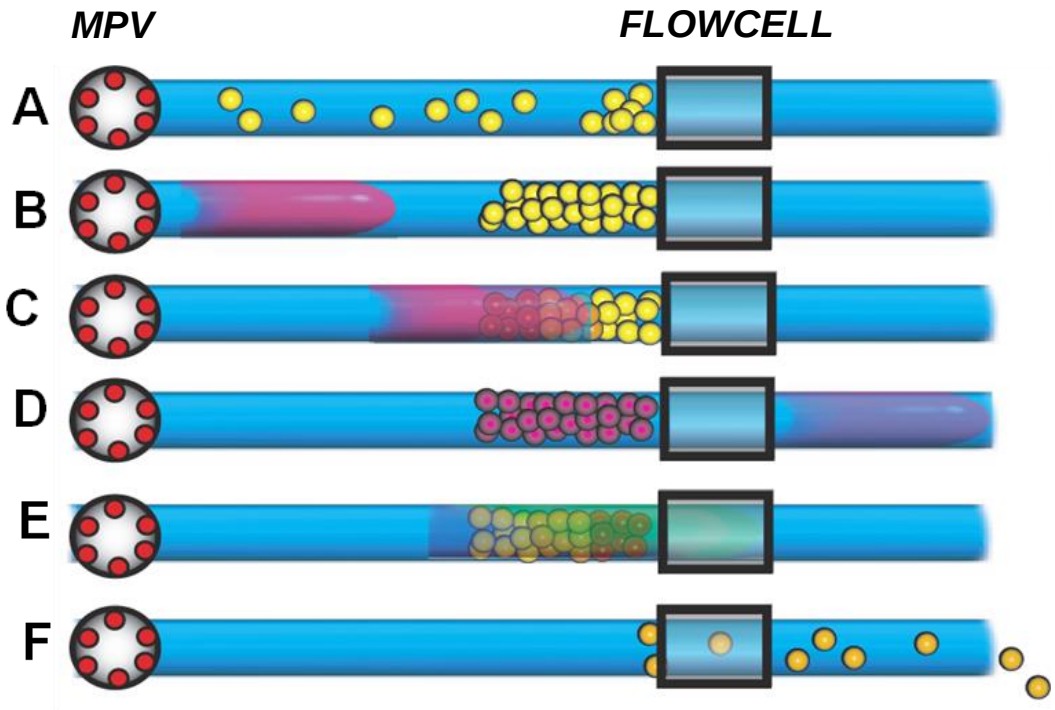
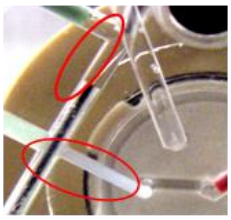
Bead Injection

BI exploits flow programming to meter, transport, capture, perfuse, monitor and discharge microspheres in order to separate and assay species that can be bound and released from immobilized functional groups. BI uses microliter volumes of Sepharose or other chromatographic materials available with a wide variety of functional groups. The BI technique can be configured in two ways: as a microSI chromatography or as BI spectroscopy.
BI opens unexplored avenues for development of novel (bio)analytical assays, for research in solid-liquid interactions, for study of quality of chromatographic supports, and for optimal of immobilization of bioligands on solid supports. BI is far more versatile and less expensive than analytical methods based on the use magnetic beads.

MicroSI Chromatography on Renewable Column

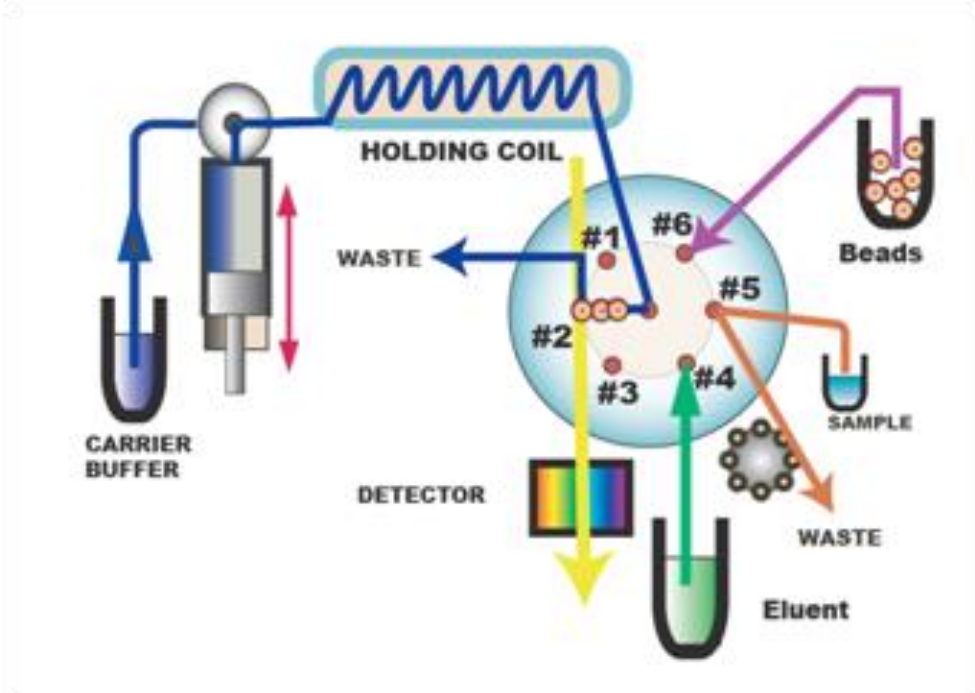


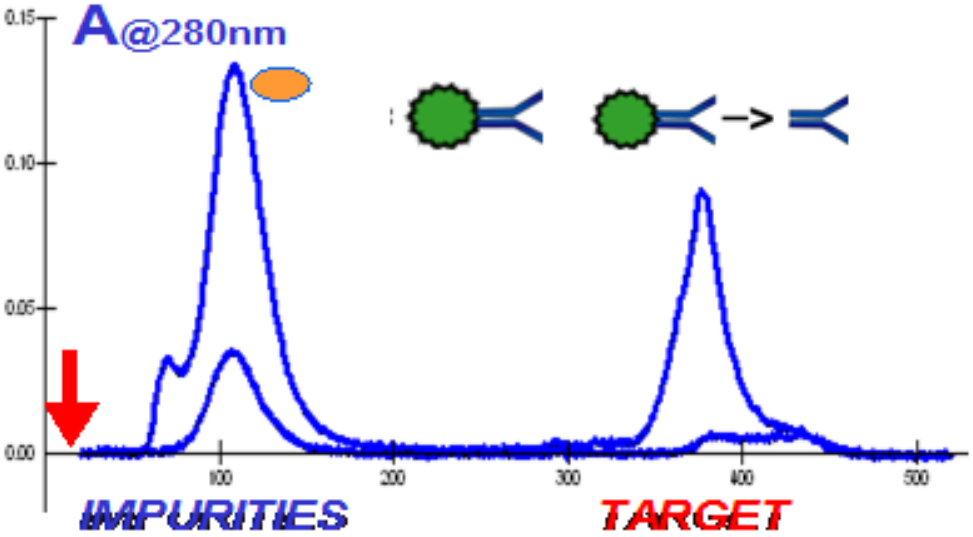

COLUMN

The assay protocol for microSIC has the following steps:

A) A precise volume of bead suspension is aspirated via MPV, and by flow reversal dispensed downstream where the beads are captured in a well-defined geometry in a close proximity to the flow cell (see insert).
B) The MPV is switched towards the sample source, a precise volume of sample solution is aspirated, and after MPV is switched towards the column, the flow is reversed.
C) The injected sample zone passes through the column where the analytes are retained.
D) The matrix as well as any not retained components are washed out.
E) A precise volume of eluent is aspirated, the MPV is switched and the eluent is directed trough the column. The eluted analyte is monitored as it passes through the flow cell
F) The microcolumn is discarded.

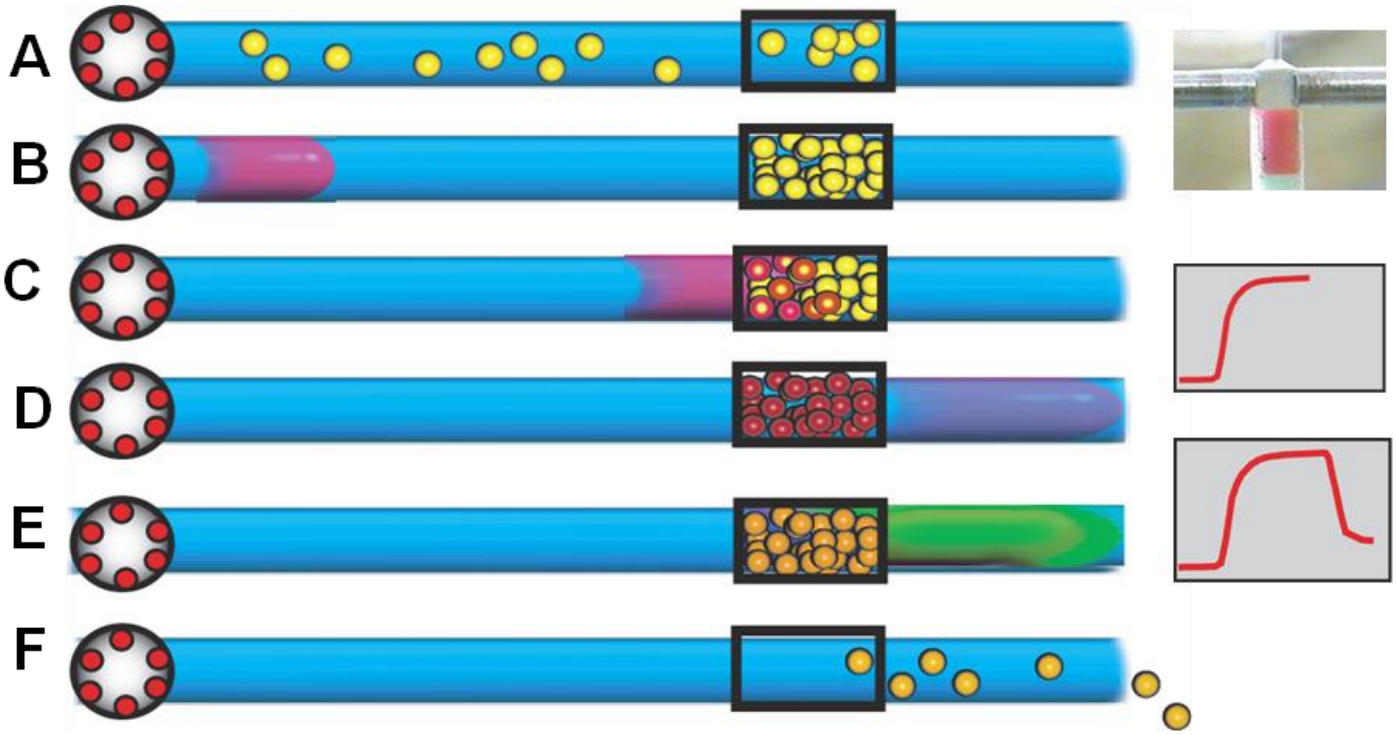
Example: MicroAffinity Chromatography





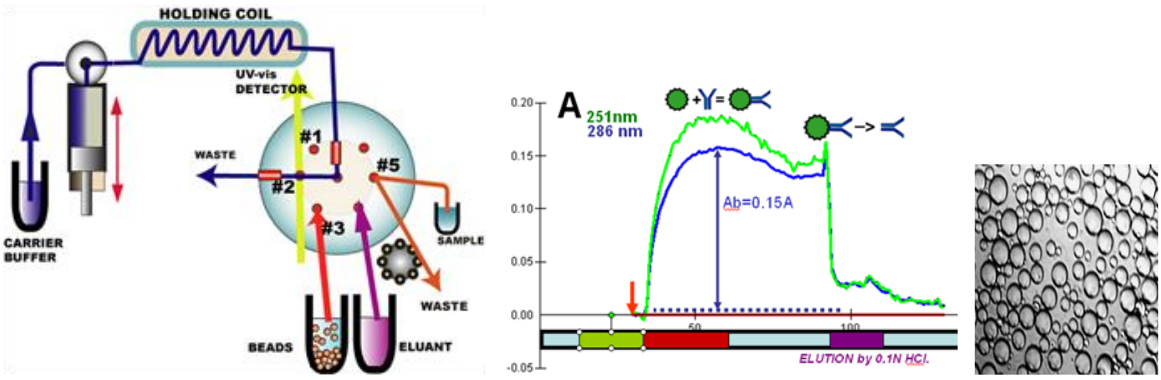
Quality control of Monoclonal Antibodies is carried out in real time on Protein A or Protein G Sepharose 6B microcolumns. Separation and assay is completed within 120 to 240 seconds, with detection limit of 6ng mouse IgG, monitored at 280nm. Target analytes are eluted from a microcolumn by pulses of eluant and monitored using UV-VIS spectrometry for quantification of native IgG or labeled biomolecules Separation of mouse IgG from bovine serum albumin on a renewable microcolumn Protein A Sepharose 6B is shown here. For details see Tutorial.

Bead Injection Spectroscopy



The assay protocol for BI Spectroscopy has the following steps:
A) A precise volume of bead suspension is aspirated via MPV, and by flow reversal dispensed downstream, where the beads are captured in a well-defined geometry within the flow cell (see insert).
B) The MPV is switched towards the sample source; a precise volume of sample solution is aspirated, and after the MPV is switched towards the flow cell and the flow is reversed.
C) The injected sample zone passes through the flow cell, where the analytes are retained on bead surfaces and detected. Note (insert), that the response curve has a plateau, if the retained analyte is bound firmly to bead surfaces.
D) The matrix, not retained components is washed out.
E) A small volume of eluent is aspirated, the MPV is switched and the eluent is directed trough the flow cell. If all retained species are eluted the signal will reach baseline. If not the beads within the flow cell can be automatically removed.
F) The beads are discarded from the flowcell.

Example: BI Spectrosopy of Native Insulin



Sephadex Beads

Selective capture of native insulin antibody and its nonselective elution by a 25 mcrL pulse of 0.1 N HCl has been monitored at 251 and 286 nm. Sephadex-Protein A beads. For details see Tutorial.

Beads

Sephadex and Sepharose beads are ideal for BI applications since they are transparent allowing target molecules to be monitored by UV-VIS and by fluorescence. These beads are available with a wide variety of functional groups: cation and anion exchange, C-18, Protein A, Protein G , avidin and streptavidin. They vary in diameter between 30 and 150 micrometers.