24 SAMPLES AT A TIME
8 RUNS PER DAY

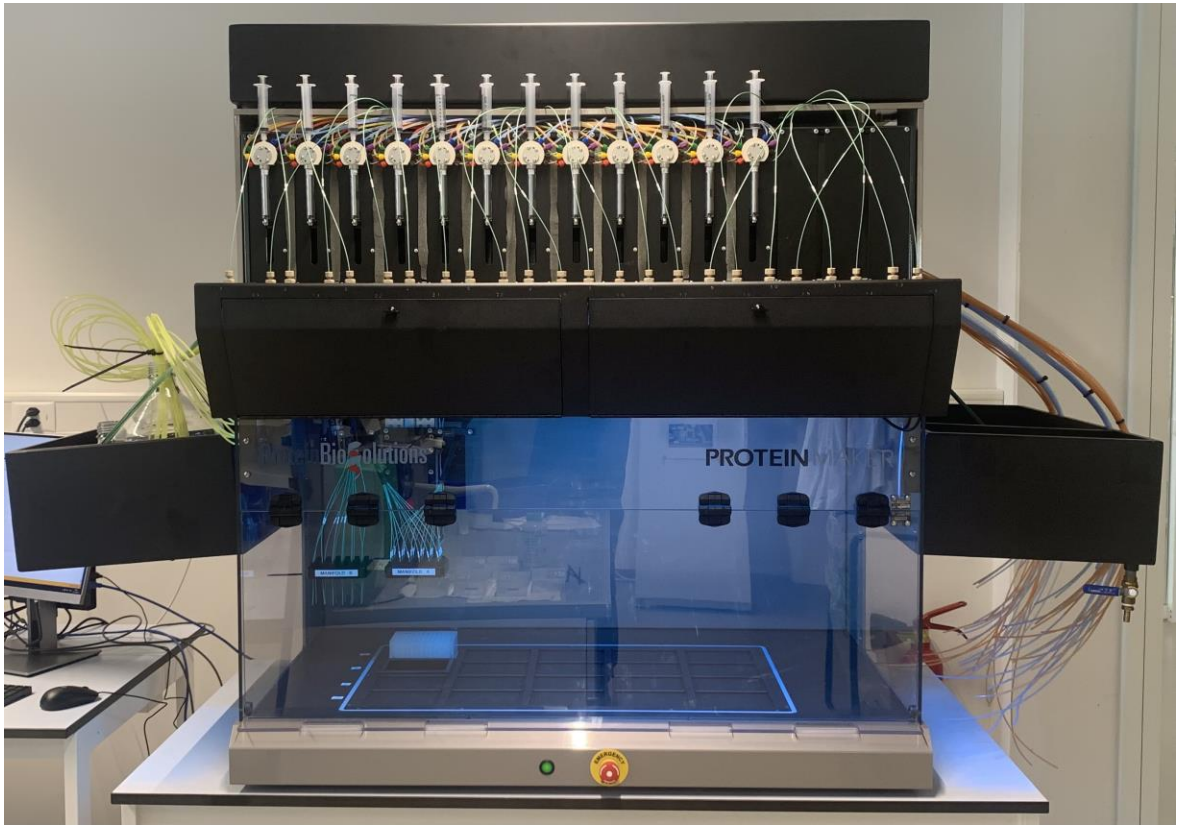
RELIEVE YOUR BOTTLENECKS

192 PURIFICATIONS
PER DAY

ProteinBioSolutions

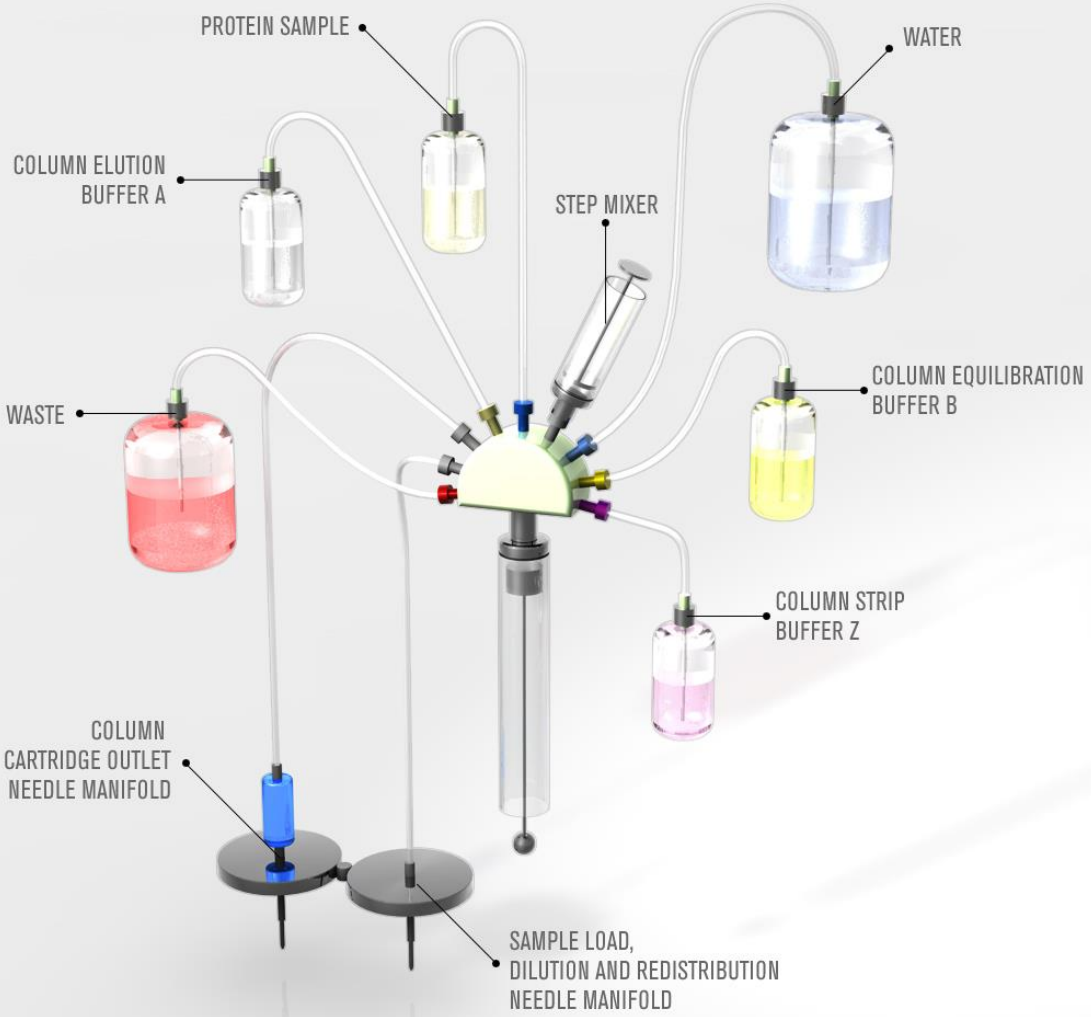
Overview

- Purify 1 to 24 samples in parallel
- Eliminate known and unknown (!) sample degradation
- mg+ protein production scale
- Flexible sample volume scales from a few mL to multiple liters



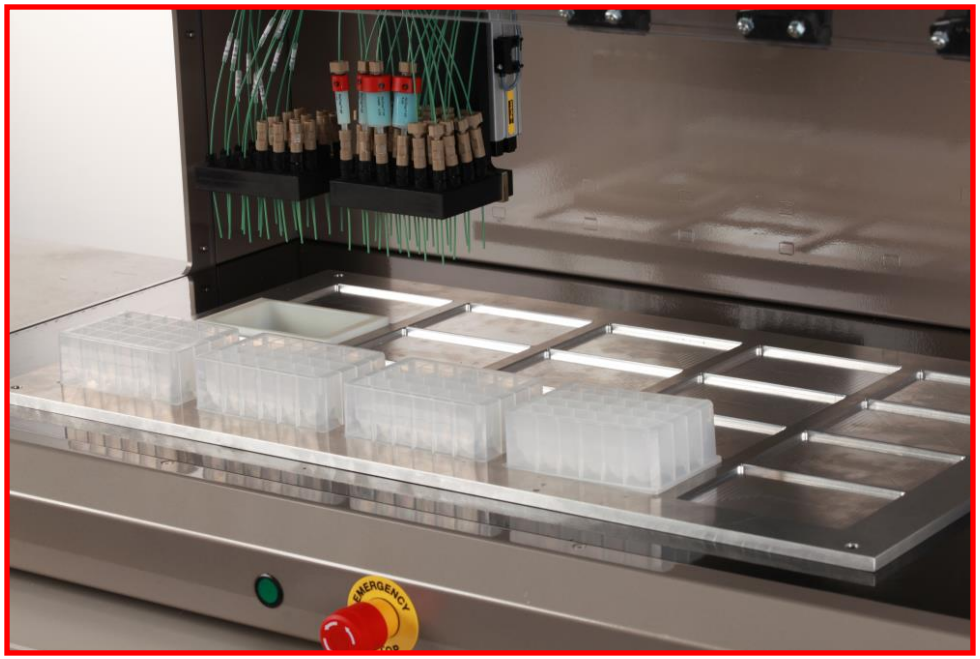
Overview

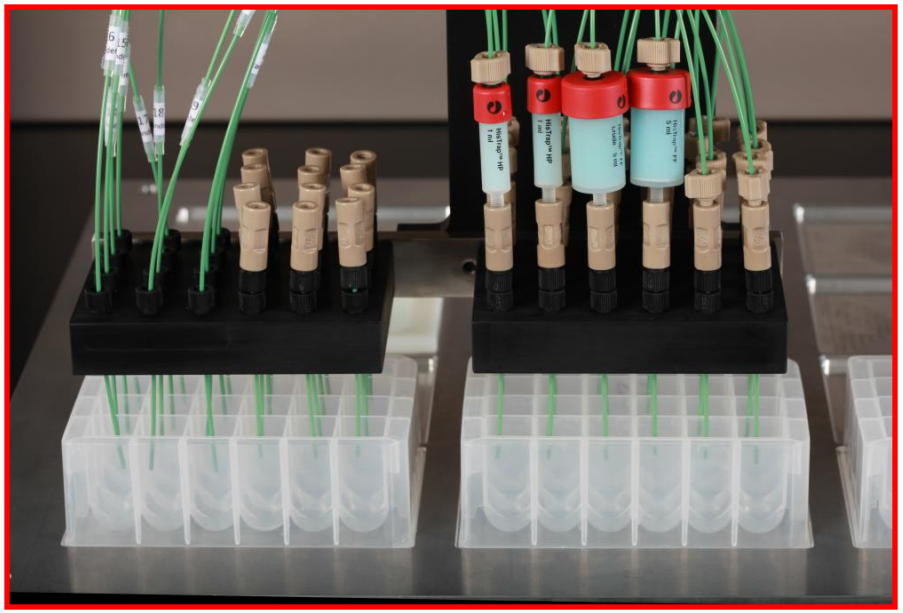
- 24 independent flow paths
- 9-port valve configuration
- Compatible with common commercial columns



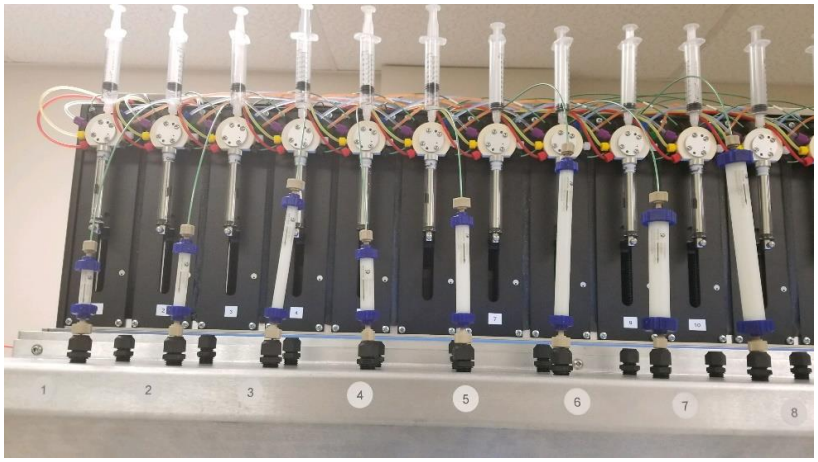
Overview

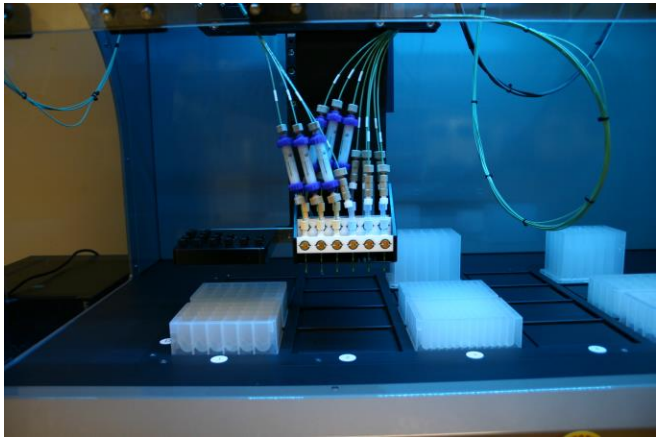
- 20 SBS deck positions (1 waste)
- Walk-away automation
- Perform parallel, 2-step purification of 12 samples



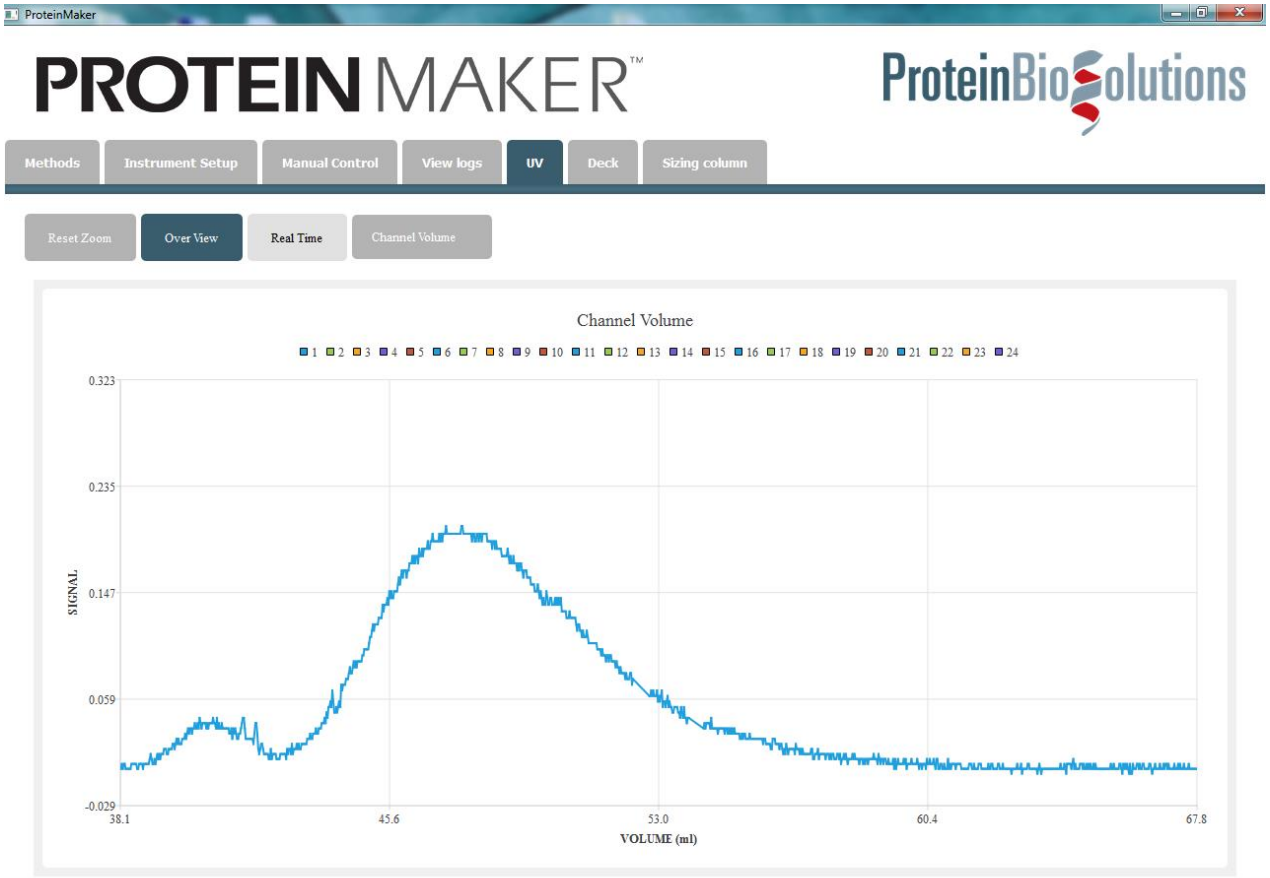


Flexible column sizes and locations

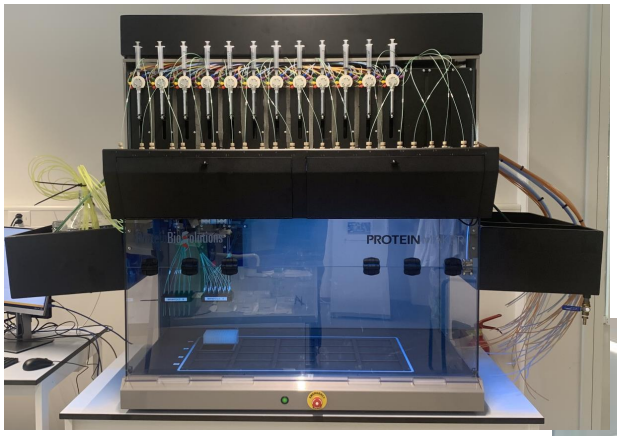




Now with optional 24 channel UV monitoring system!



Individual UV Flow cell block for each of 24 channels:



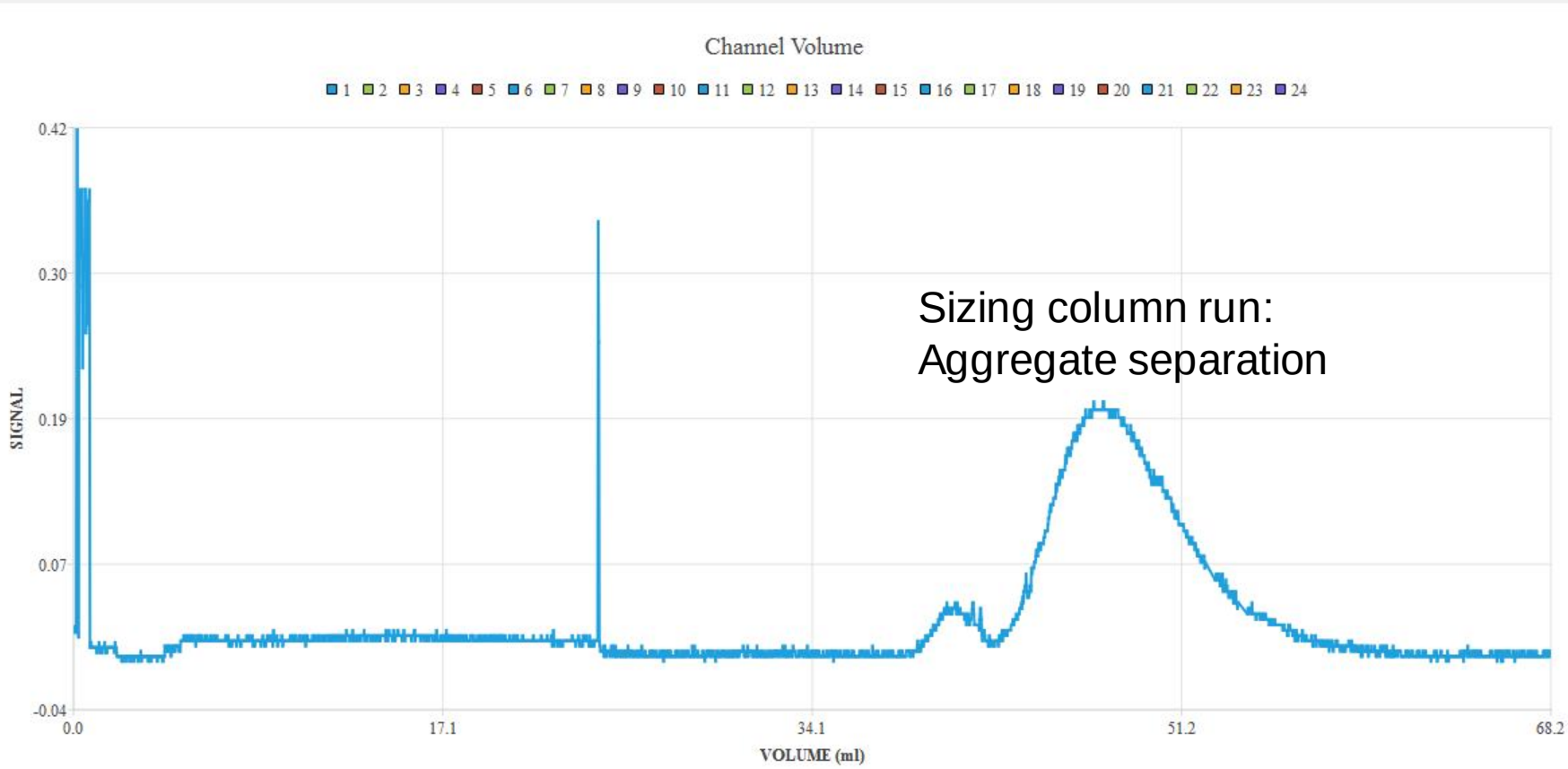
ProteinMaker

PROTEIN MAKER™

ProteinBioSolutions

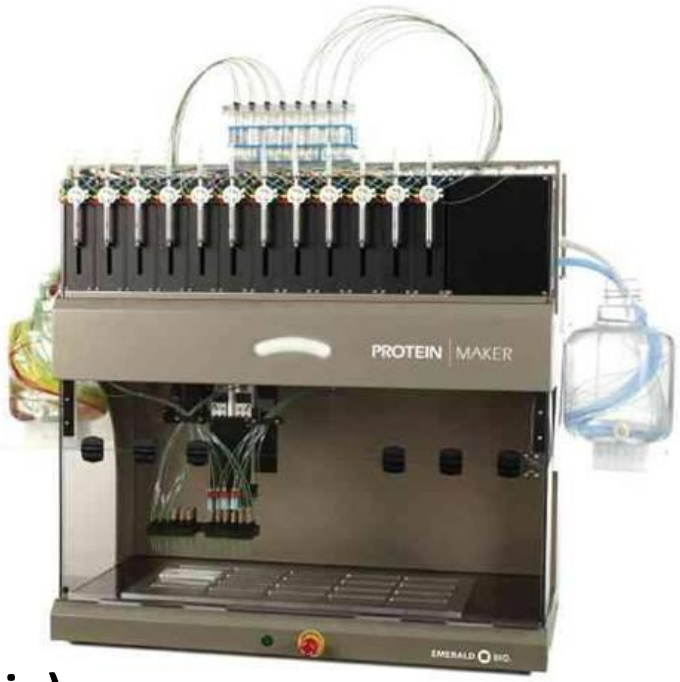
Methods | Instrument Setup | Manual Control | View logs | UV | Deck | Sizing column

Reset Zoom | Over View | Real Time | Channel Volume



Protein Maker™: Purification Automation

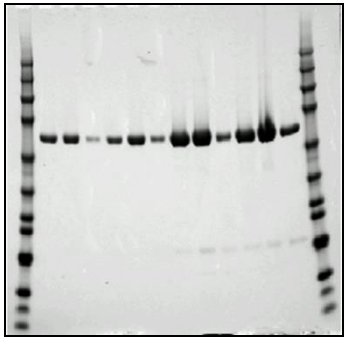
- High-throughput Protein Purification



- **Operation Modes:**
  1. **Production Mode (up to 24 proteins, 1 resin)**
  2. **Scouting Mode (up to 24 resin types, 1 protein)**
  3. **Multi-column (automated 2 step purification)**

- **Common Applications / Uses:**
  1. **Antibody Production**
  2. **Crystallography Prep**
  3. **No Risk Scale-up**

12 protein variants purified in parallel



Production Mode

<u>Antibody production/screening:</u>
Purify mg+ amounts of up to 24 antibodies in parallel using up to or more than 1L

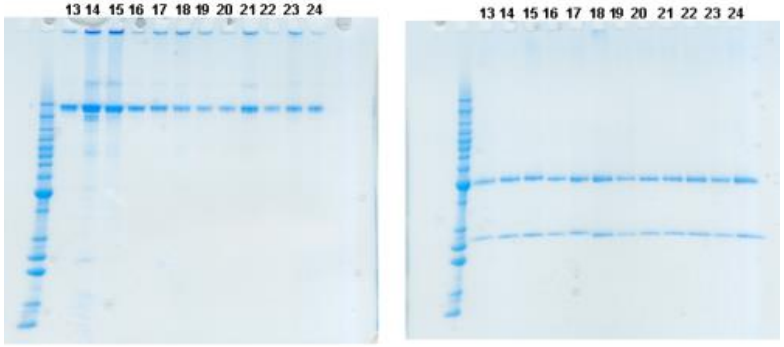
Case Study: Purification of Engineered Antibodies

- Had: Fully humanized antibody against a viral antigen target, with high affinity for wild type target but no affinity to a prevalent naturally occurring variant

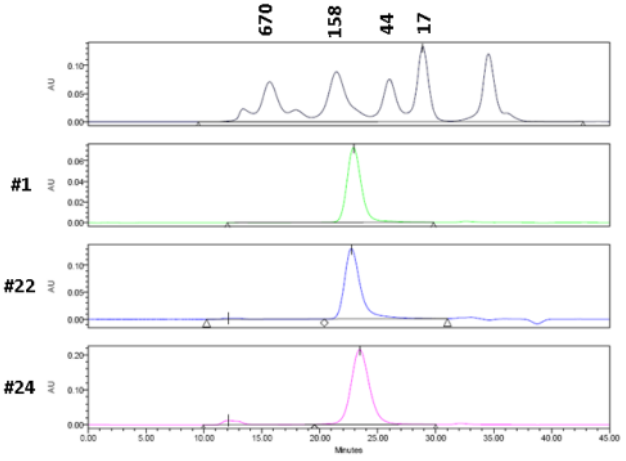
- Goal: improve affinity for variant without loss of affinity for wild type

Purified 50mL each for 24 mutations (expression in CHO cells) using 1 mL HiTrap Protein A column. Yield (2-10 mg) provided sufficient material for biophysical characterization (analytical SEC/LS, SDS-PAGE, DSC) as well as affinity measurement.

Example SDS-PAGE: Mutants 13-24



Example analytical SEC: Mutants 1, 22, and 24



Production Mode

Production of multiple constructs:

Parallel processing enables scaled-up purification of multiple constructs (internal deletion variants) to access those with high crystallizability

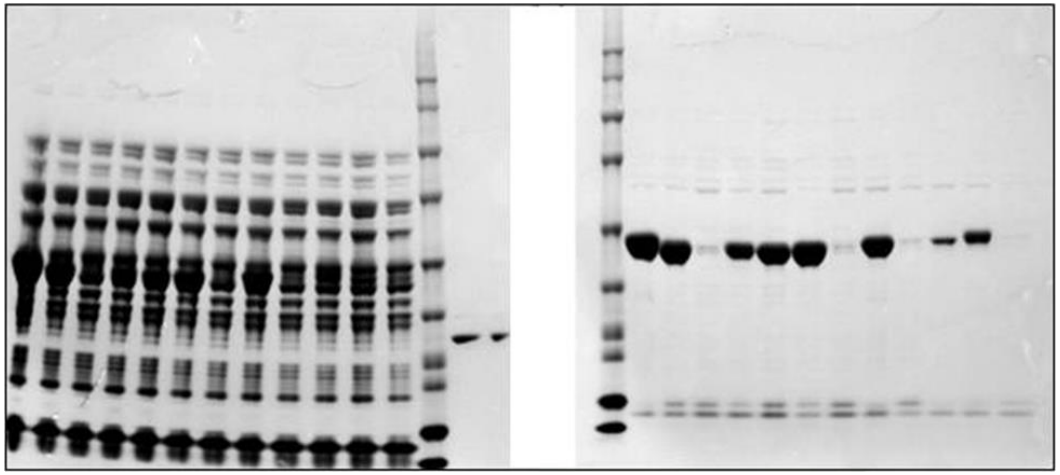
Case Study: Multi-construct design and purification

Lysates

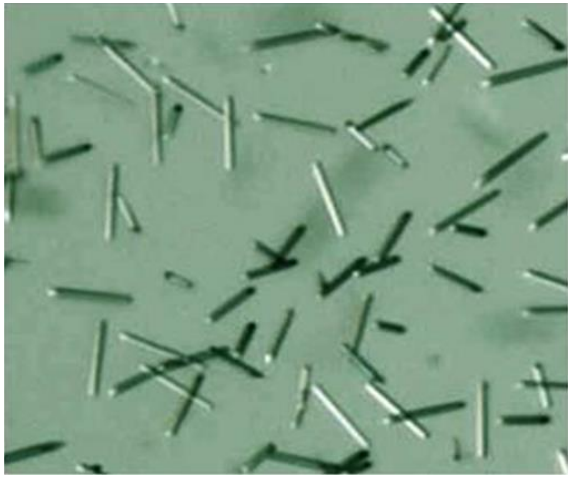
1 2 3 4 5 6 7 8 9 10 11 12

Eluates

1 2 3 4 5 6 7 8 9 10 11 12



Crystals



Genes optimized for *E. coli* expression

Expression yields: 0.1 to 15mg per liter cell culture

Production Mode

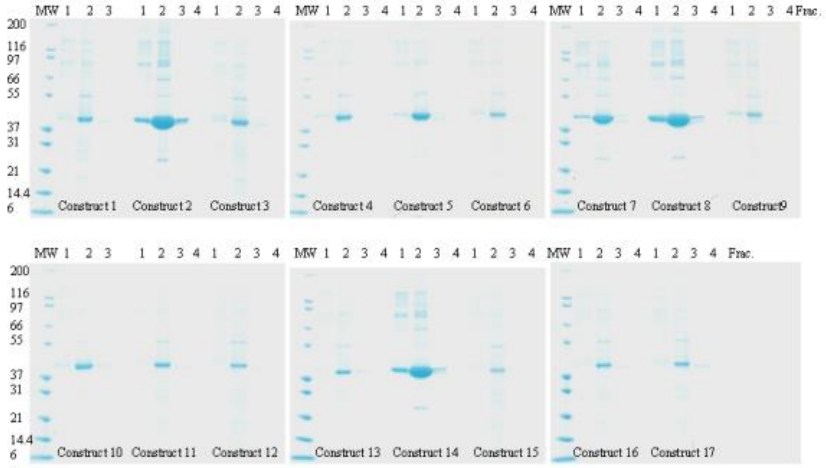
<u>Production of multiple constructs:</u>

Parallel processing enables more efficient route to protein crystals

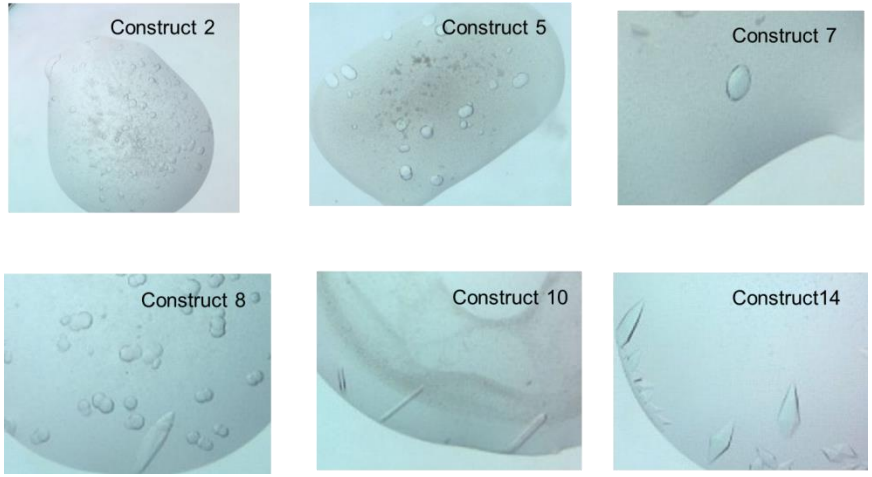
Case Study: Production of Kinase X – 17 constructs

Parallel nickel affinity purification

(4-fraction elution)



9 constructs selected for his-tag removal and crystal screening



Production Mode

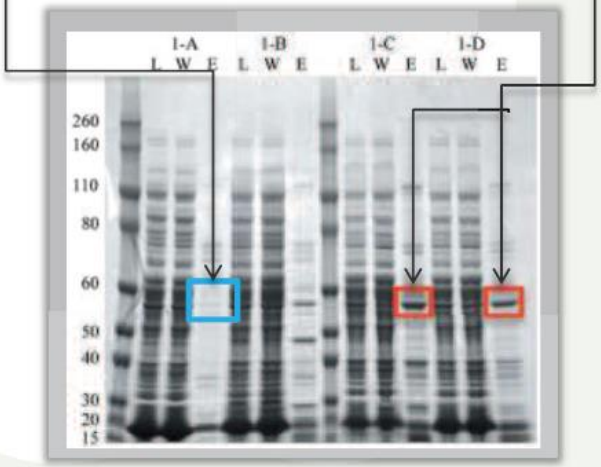
Parallel Lysis scouting:

Test 12 different lysis buffer conditions followed by small scale IMAC purification to scale up and move forward with the strong purifiers

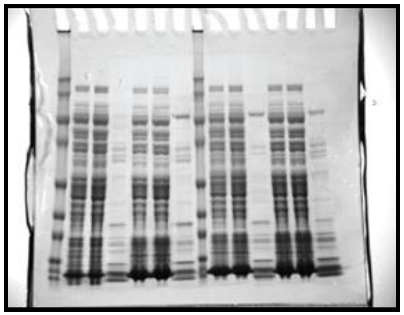
Case Study: Parallel testing of Lysis Conditions

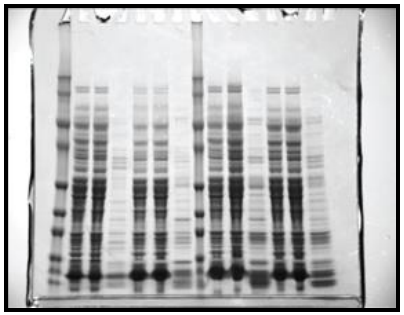
Example: Cytochrome P450 (CYP51A) insoluble in standard lysis buffer

Result: of 12 lysis buffers 2 yield soluble CYP51A (CHAPS/bOG + 500 mM NaCl)



Conditions:	Low Salt	High Salt	Detergent 1	Detergent 2
pH 6.0	50mM MES 250mM NaCl 5% Glycerol 0.5mM TCEP	50mM MES 1M NaCl 5% Glycerol 0.5mM TCEP	50mM MES 500mM NaCl 5% Glycerol 0.5mM TCEP 1% CHAPS	50mM MES 500mM NaCl 5% Glycerol 0.5mM TCEP 1% BOG
pH 7.5	50mM HEPES 250mM NaCl 5% Glycerol 0.5mM TCEP	50mM HEPES 1M NaCl 5% Glycerol 0.5mM TCEP	50mM HEPES 500mM NaCl 5% Glycerol 0.5mM TCEP 1% CHAPS	50mM HEPES 500mM NaCl 5% Glycerol 0.5mM TCEP 1% BOG
pH 8.0	50mM TRIS 250mM NaCl 5% Glycerol 0.5mM TCEP	50mM TRIS 1M NaCl 5% Glycerol 0.5mM TCEP	50mM TRIS 500mM NaCl 5% Glycerol 0.5mM TCEP 1% CHAPS	50mM TRIS 500mM NaCl 5% Glycerol 0.5mM TCEP 1% BOG





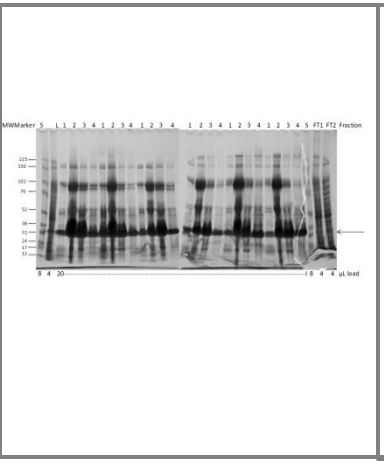
Acta Cryst. (2011). F**67**, 1015–1021

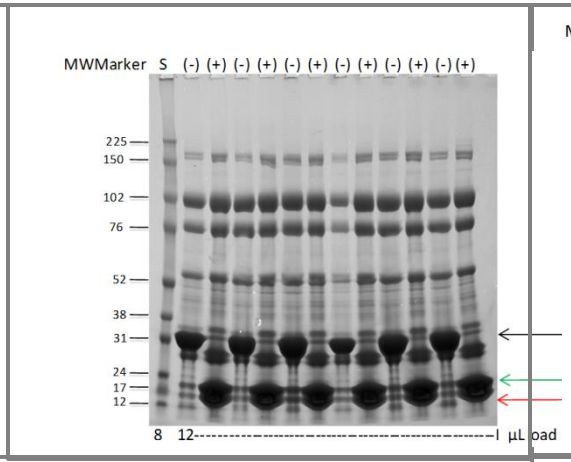
Production Mode

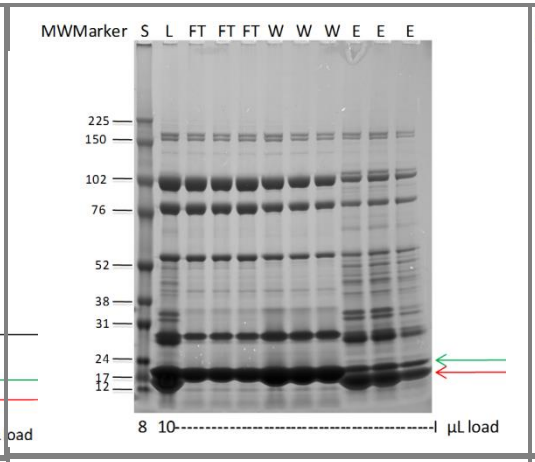
Risk-free Scale-up via parallelization:

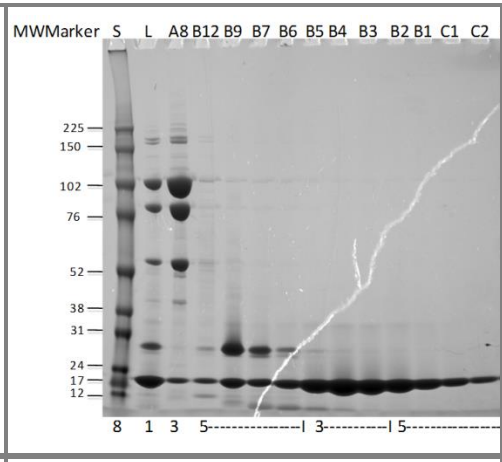
6 x 5 mL Column Bed Volume Parallel Purification

Ni-affinity Chromatography → Fusion Protein Cleavage → 2nd Ni-affinity Chromatography → Size Exclusion Chromatography







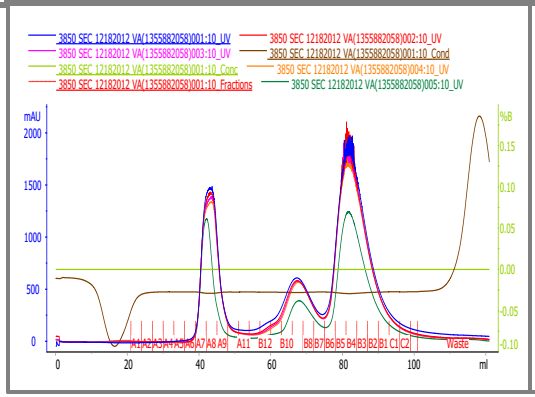


Situation

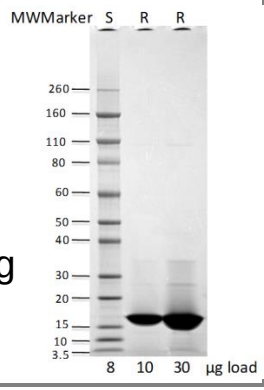
- Increased purification from 20 mg to > 100 mg

Options

- Develop protocol for 5 x scale up
- Repeat 5-6 x of the original purifications
- Run multiple small-scale purifications in parallel – Protein Maker



Yield: 176 mg
> 95% purity



No need for traditional "scale-up" if you use Protein Maker

Scouting Mode

Parallel resin scouting:

Scouting a variety of resins (up to 24) for optimization of 1 protein

Case Study: Scouting Mode with a Step-Gradient Protocol

Glu-PGS – Antibody affinity column
A. Equilibration Buffer: 20 mM Tris pH 8, 100mM NaCl, 0.5% NP40
B. Elution Buffer: Equilibration buffer 1 plus 50 μM EYMPTD peptide

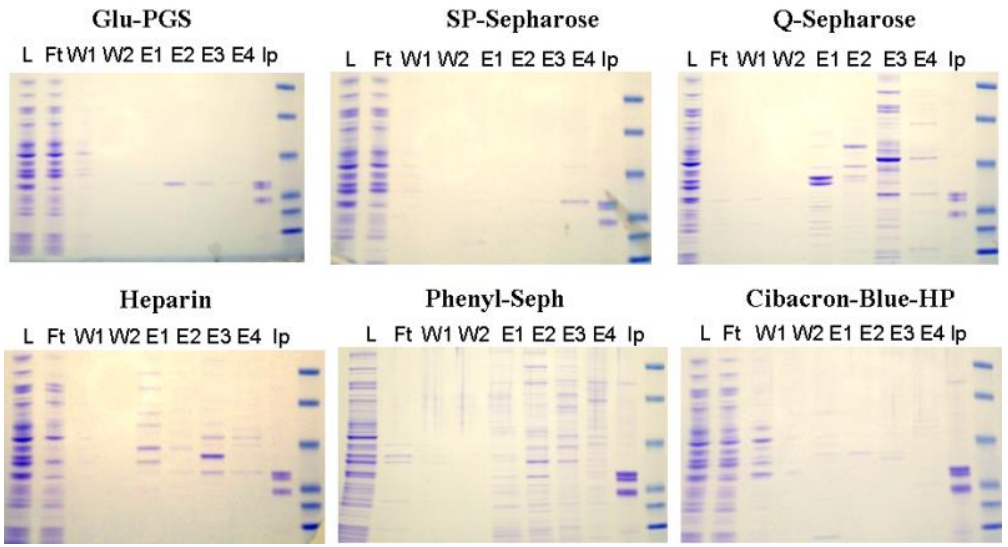
HiTrap SP Sepharose – Cation exchange resin
A. Equilibration Buffer: 20 mM MES pH 6.0
B. Elution Buffer: Equilibration buffer plus 1 M NaCl

HiTrap Q Sepharose – Anion exchange resin
A. Equilibration Buffer: 20 mM Tris pH 8
B. Elution Buffer: Equilibration buffer 1 plus 1 M NaCl

Heparin Sepharose – Cation exchange resin
A. Equilibration Buffer: 20 mM MES pH 6.0
B. Elution Buffer: Equilibration buffer plus 1 M NaCl

HiTrap Phenyl Sepharose – hydrophobic interaction
A. Equilibration Buffer: 20 mM Tris pH 8, 1M NH4SO4
B. Elution Buffer: 5 mM Tris pH 8

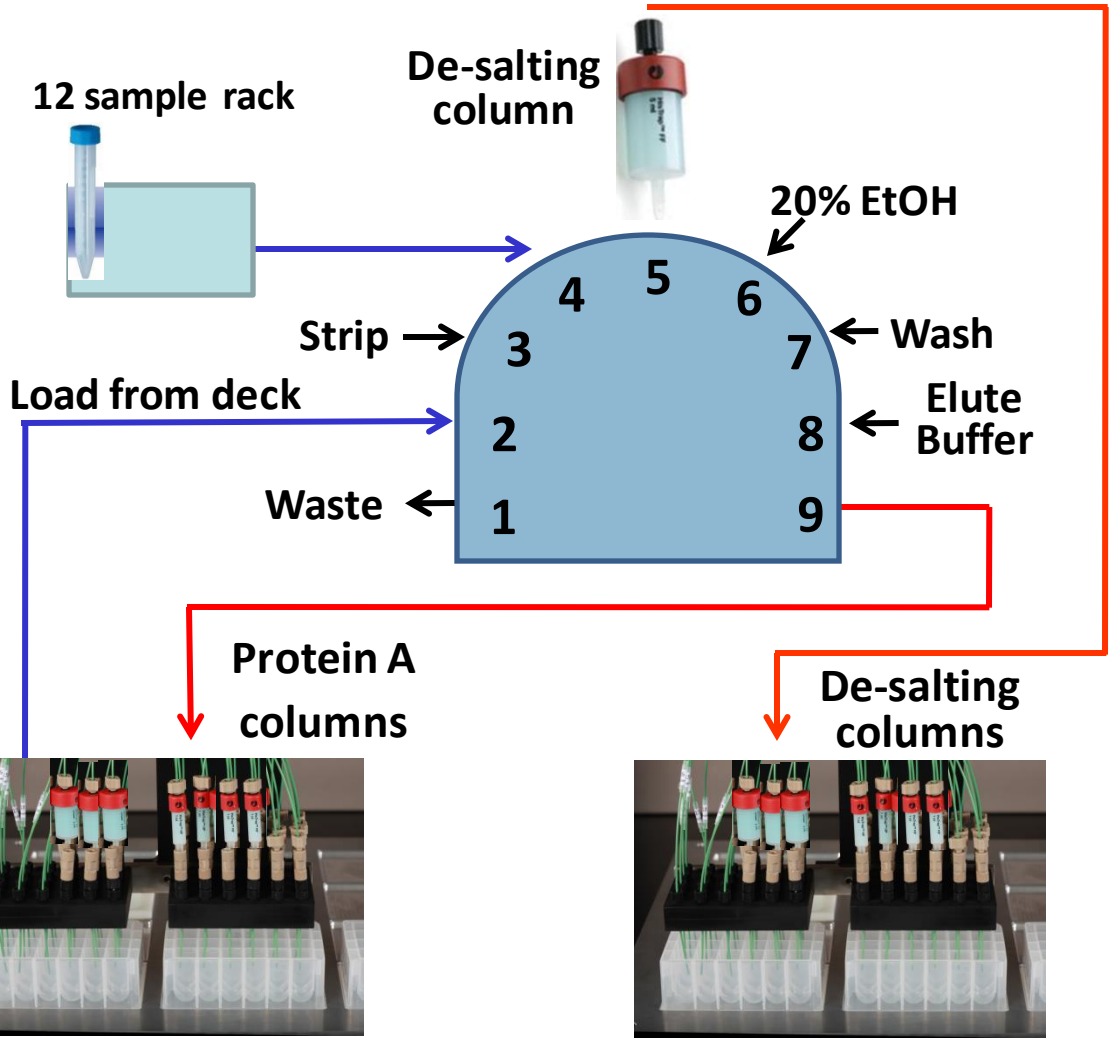
HiTrap Blue Sepharose - affinity column
A. Equilibration Buffer: 20 mM Tris pH 8
B. Elution Buffer: Equilibration buffer 1 plus 1 M NaCl



Multi-Column purification

2-column Purification Schematic:

- 12 Protein A columns (1 ml)
- 12 Desalting columns (5 ml)
- Load volume: 50 ml (from sample rack)
- Run time: 2.5 h unattended operation
- Run includes column regeneration



Complex Purification Mode

2-step Purification:

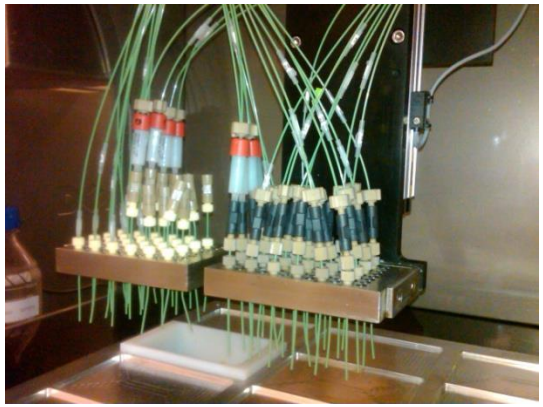
Ni and Protein A 2-step Fab purification

Case Study: Need medium scale throughput of 2-step protocol

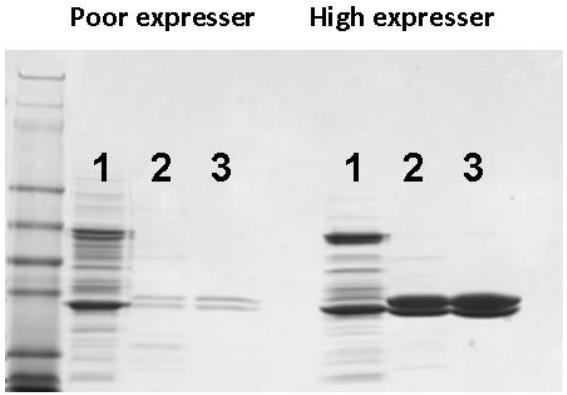
- Ni purification (GE His-Trap FF) of His-tagged Fabs alone requires additional purification, particularly for low-producing proteins, but generally results in high yield
- Ni requires buffer exchange post purification
- Protein A (GE Hi-Trap FF) is added in series to increase purity and remove requirement for buffer exchange

Customized Potential Protocol:

- Syringes 1-12: Load Ni, wash, and elute with 200 mM imidazole-containing buffer
- Syinges 13-24: Load Ni eluate, wash, elute with pH 2.8 buffer with immediate post-column neutralization



Syringes 1-4 His-Trap FF (right); syringes 13-16 Hi-Trap Protein A FF (left)



1: cell-free extract
2: Ni-purified
3: Ni and Protein A-purified

Customer Feedback

OPPORTUNITY COSTS

Make the best use of your time, money and equipment: *"The Protein Maker™ enables us to make the best use of our time and purification equipment by saving our single channel systems for complex purification experiments while the Protein Maker™ quickly works through our routine and/or high-throughput purifications."*

Shrink your project timelines: *"The parallelization of the Protein Maker™ enables one technician to purify more protein constructs in one day than could be done in a week by that same person. The practical result is that we can isolate the desired protein construct in four weeks faster on average for every project. That is extremely valuable!"*

Enable new strategies: *"Parallel purification of 12-24 protein samples enables new and otherwise impractical screening and scouting techniques that we wouldn't attempt without the Protein Maker™ and have lead to key successes."*

INTANGIBLES

Pleasing your colleagues, clients and project leaders: *"Just knowing that we have this capability in house gives me the confidence to tell the end users of the proteins we produce that I can get them their pure protein in two weeks…when they would expect it to take six or more."*