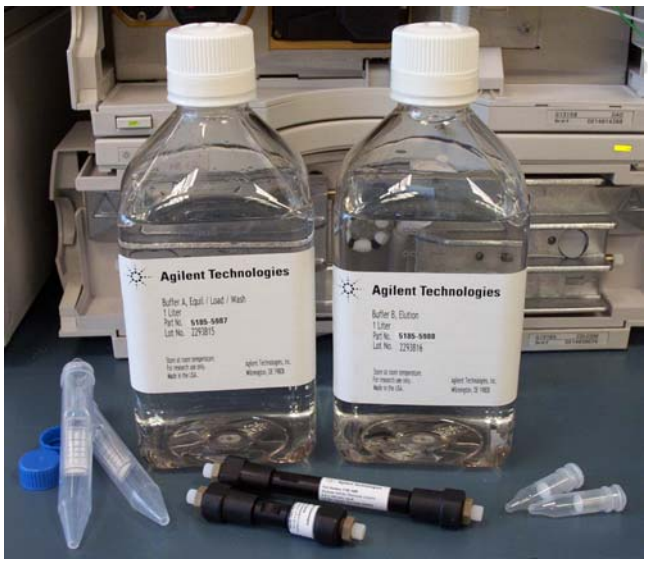
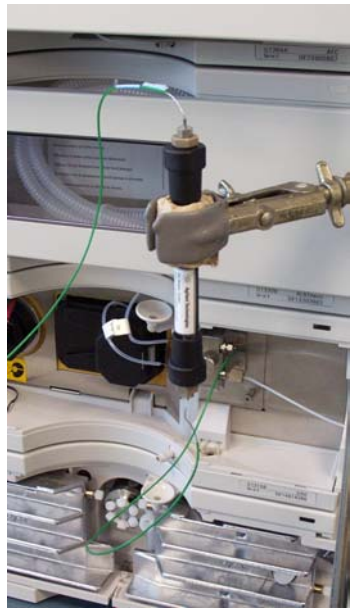
Multiple High-Abundant Protein Removal for Proteomics

Dr. Cory Szafranski, Product Manager



Agilent Technologies

Agilent Multiple Affinity Removal System - What is it?

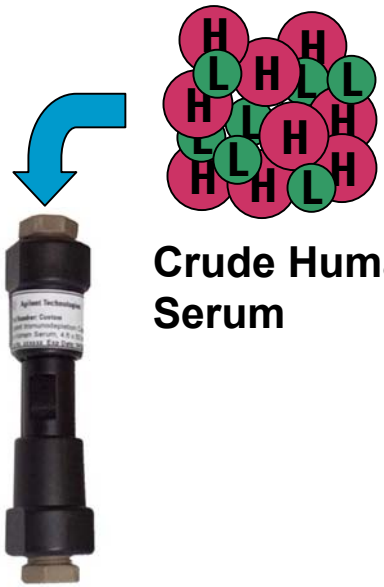


H High-Abundant Proteins (Albumin, IgG, IgA, Transferrin, Haptoglobin, Antitrypsin)

L Low-Abundant Proteins (Biomarkers for disease and drug targets)

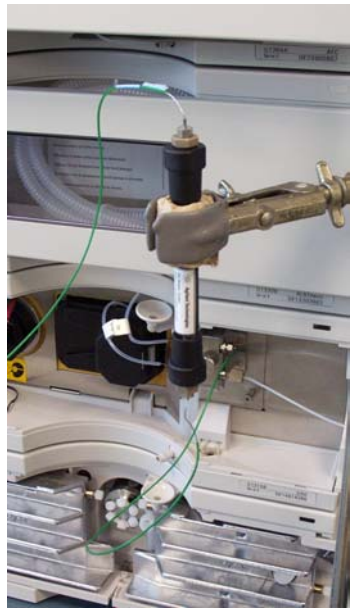
- Column and Optimized buffers are used to remove the top six most abundant proteins in human serum and plasma samples.
- Attach to HPLC instrument and pump samples through - proteins of interest are collected and analyzed.

Agilent Multiple Affinity Removal System - What is it?



H High-Abundant Proteins (Albumin, IgG, IgA, Transferrin, Haptoglobin, Antitrypsin)

L Low-Abundant Proteins (Biomarkers for disease and drug targets)

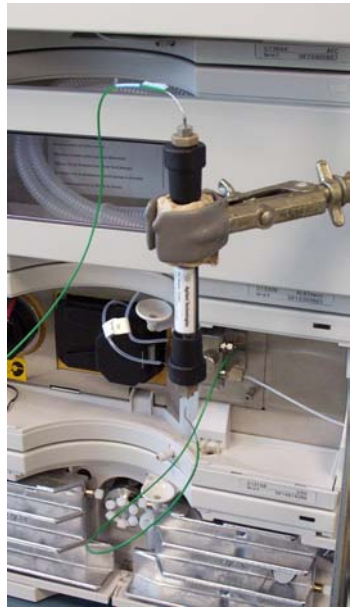


- Column and Optimized buffers are used to remove the top six most abundant proteins in human serum and plasma samples.
- Attach to HPLC instrument and pump samples through - proteins of interest are collected and analyzed.

Agilent Multiple Affinity Removal System - What is it?

H High-Abundant Proteins (Albumin, IgG, IgA, Transferrin, Haptoglobin, Antitrypsin)

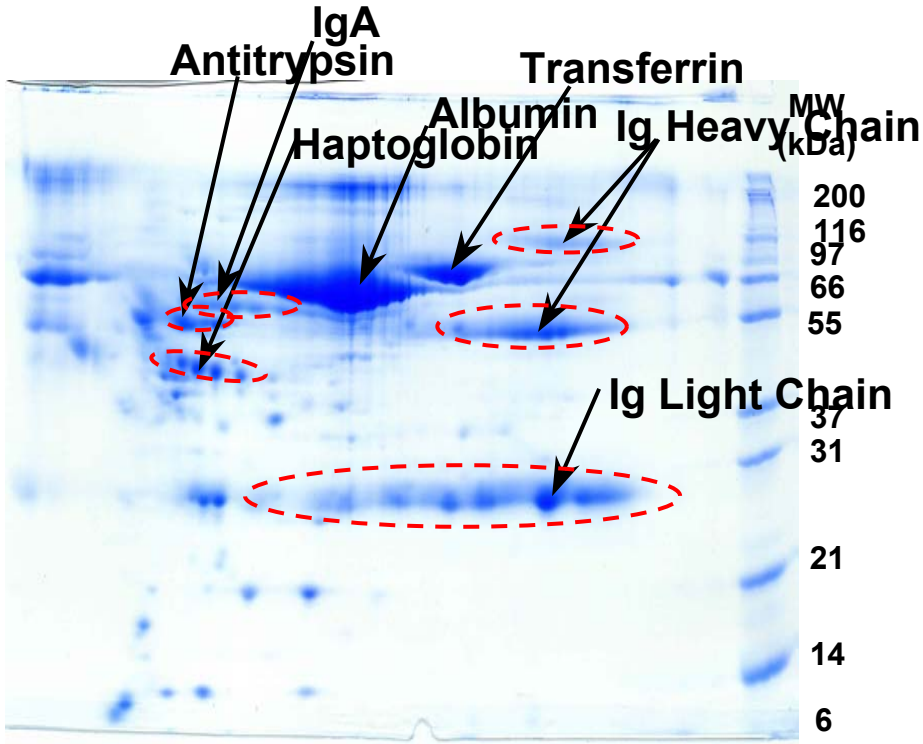
L Low-Abundant Proteins (Biomarkers for disease and drug targets)



- Column and Optimized buffers are used to remove the top six most abundant proteins in human serum and plasma samples.
- Attach to HPLC instrument and pump samples through - proteins of interest are collected and analyzed.

2DGE Data for Human Serum

Before Removal



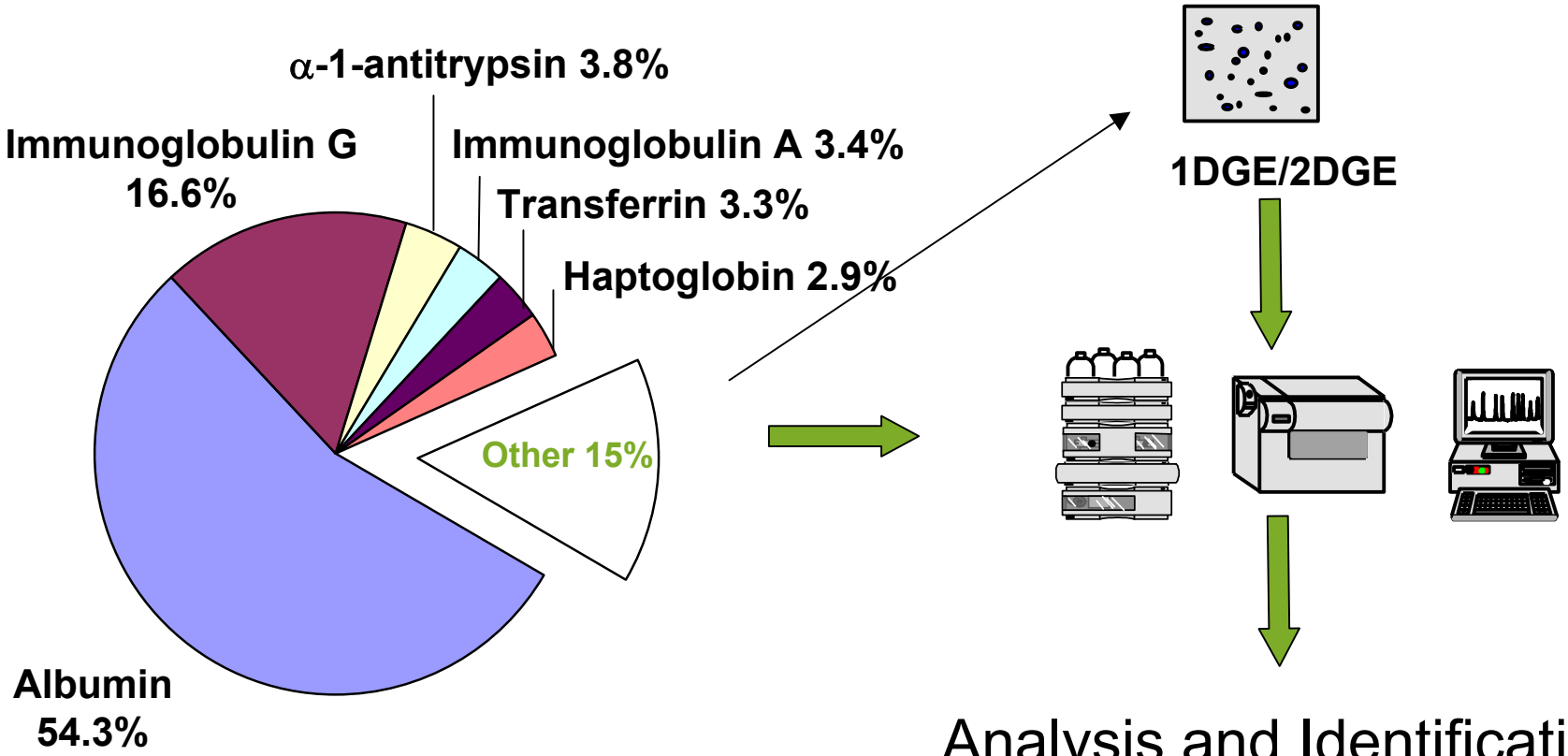
pH 3 – 10 →

250µg total protein, Coomassie Blue stained

After Removing High-Abundant Proteins

pH 3 – 10 →

High Abundance Proteins in Human Plasma



Analysis and Identification

Protein Expression
Drug Targets
Disease Markers

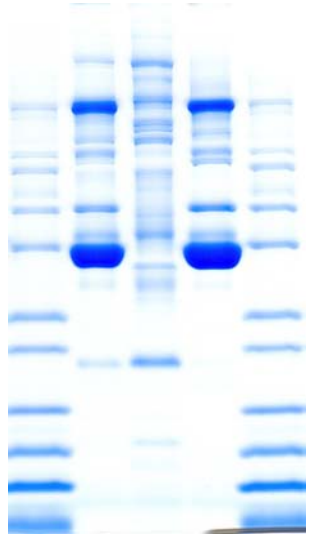
Who can benefit from this technology?

- **Biologists and Bioanalytical Scientists doing PROTEOMICS (study of the ever-changing population of proteins in the human body).**
- **Scientists trying to identify proteins in the body (serum) in very small amounts, but can't because of the presence of a few proteins that mask all of the others.**
- **Those who need to "deplete" or remove these proteins so their analytical methods can detect all of the other thousands of proteins in the sample.**
- **Those using current product or methods that do not do the job so well, and only remove one protein at a time.**

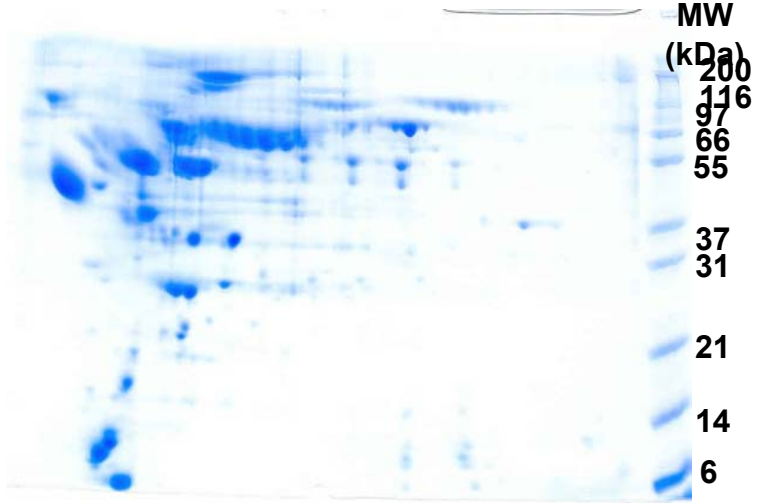
- Our product removes SIX, all at one time, and very well, and lasts for 200 injections or more!

Downstream methods for proteomics

1-Dimensional Gel Electrophoresis (1DGE)



2-Dimensional Gel Electrophoresis (2DGE)



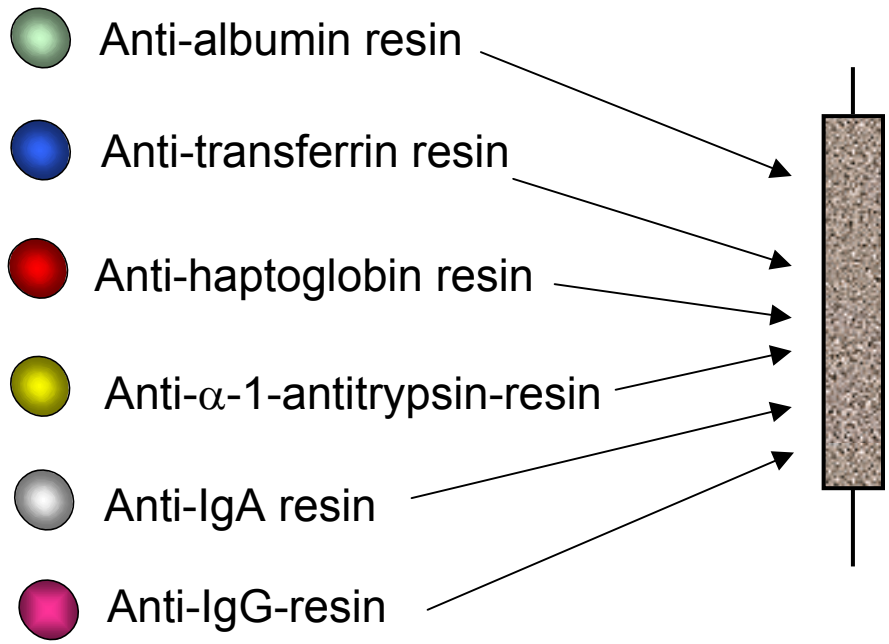
Liquid Chromatography - Mass Spectrometry (LC/MS)

Multidimensional LC (MDLC)

Bioanalyzer 2100

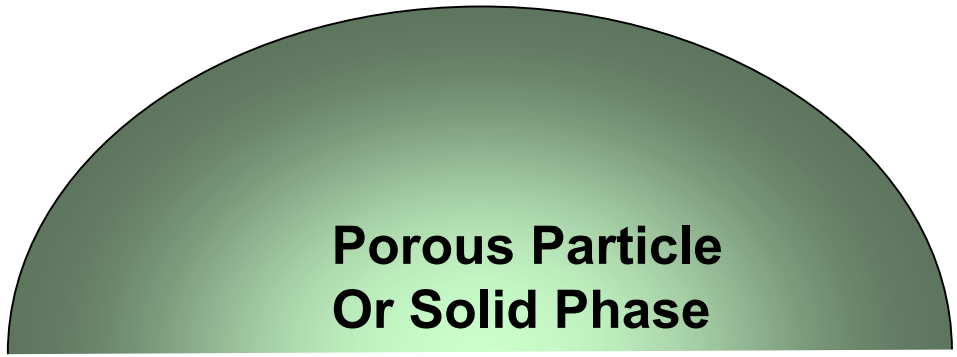
Depletion of High Abundance Proteins in Human Serum/Plasma

- **Affinity purified polyclonal antibodies bound to resin.**
- **Mixed resin bed for simultaneous removal all six proteins from serum**
- **Robust chemistry**



Individual Ab materials are mixed in selected percentages and packed into a column format.

Removal of High Abundance Proteins

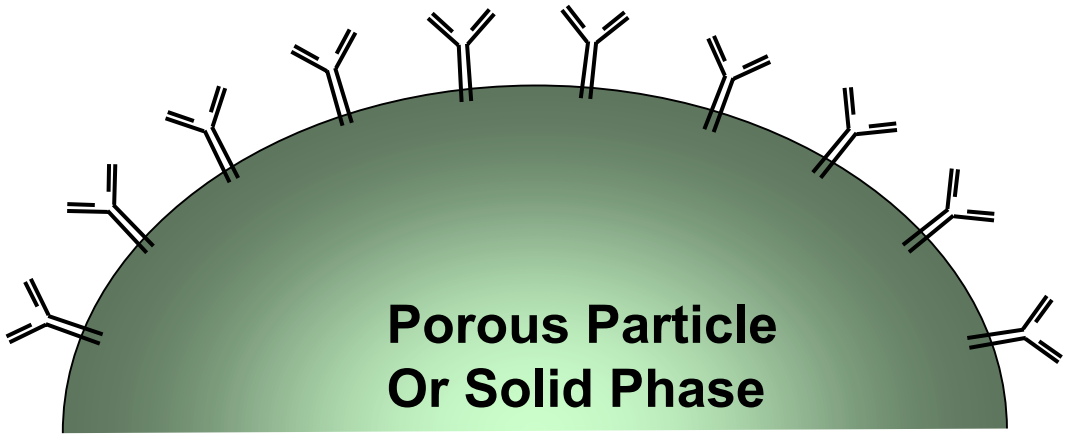


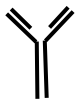
Specific Antibody

Crosslinker

Specific Target Protein (eg. Albumin)

Removal of High Abundance Proteins



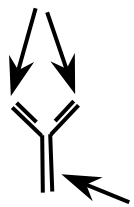


Specific Antibody

Crosslinker

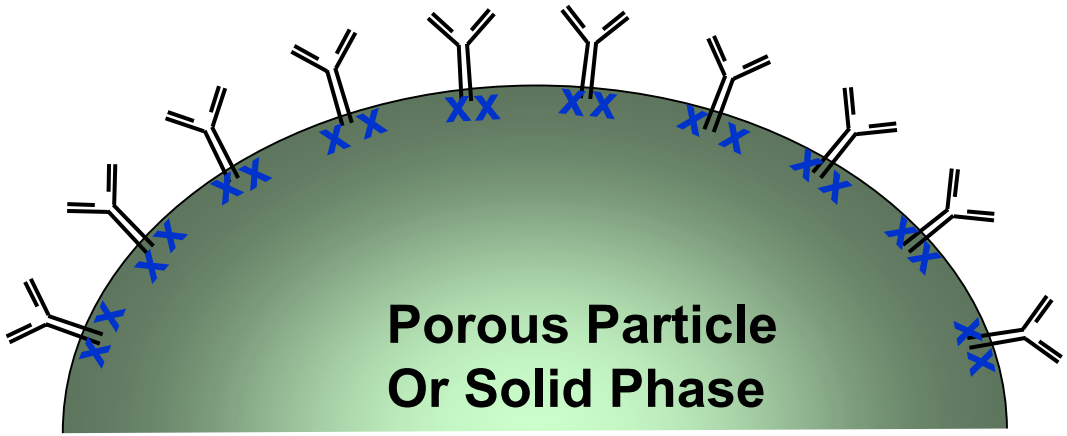
Specific Target Protein (eg. Albumin)

Antigen Binding Site



Fc Region

Removal of High Abundance Proteins

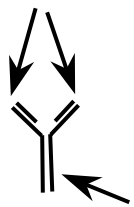


Specific Antibody

x Crosslinker

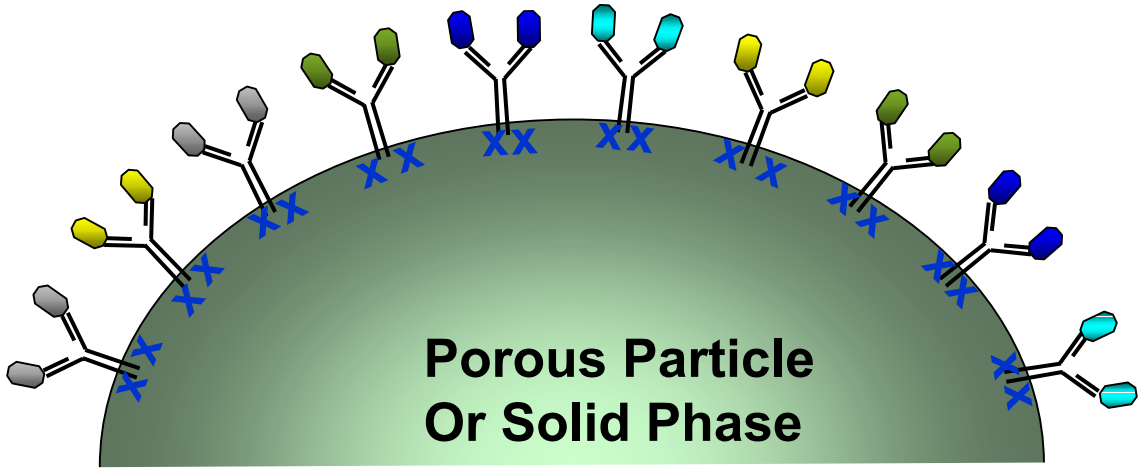
Specific Target Protein (eg. Albumin)

Antigen Binding Site



Fc Region

Removal of High Abundance Proteins



Specific Antibody

x Crosslinker

Specific Target Protein (eg. Albumin)

Antigen Binding Site

Fc Region

Columns

- Antibody-modified resins are packed into PEEK LC columns with standard 10-32 (HPLC-style) threaded column endfittings.
- Can be used on any HPLC equipment (UV detection is good for monitoring fractions coming off column.
- Maximum pressure is 120 bar.
- Flow rate range used: 0.25 - 1.0 mL/min.
- Refrigerated when not in use.

Advantages of the Technology

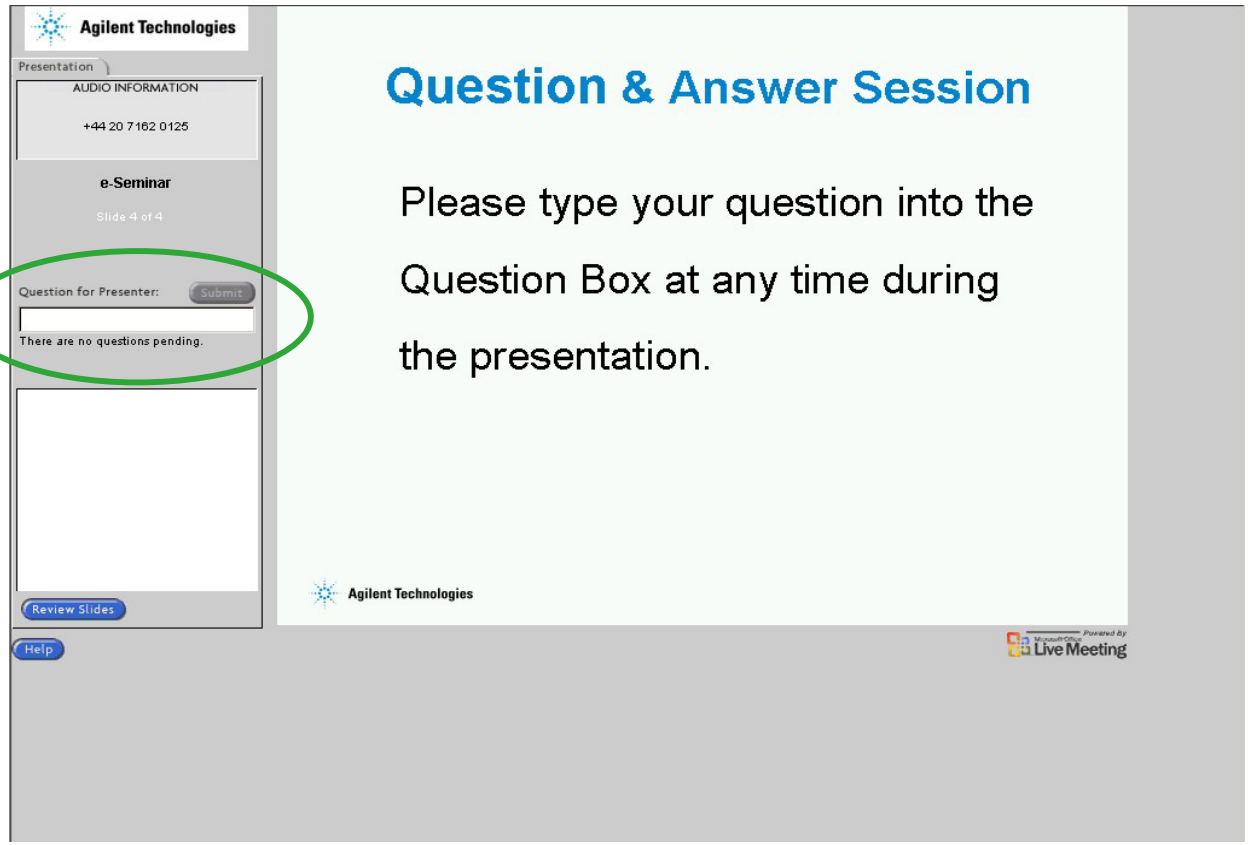
Features:

- Polyclonal antibodies bind multiple epitopes - thereby permitting depletion of proteins that may be modified due to diseases or fragmentation
- Robust chemistry enables long column lifetime and reusability
- Rapid, simultaneous removal of six proteins with one device
- Standard LC column format.

Benefits:

- Removes high-level proteins
- Unmasks lower level, rarer proteins
- Lowest non-specific binding (data to be shown)
- Fully automated with 1100 LC System

Break Number 1



Immunoaffinity Column Elution Profile - 50 mm column

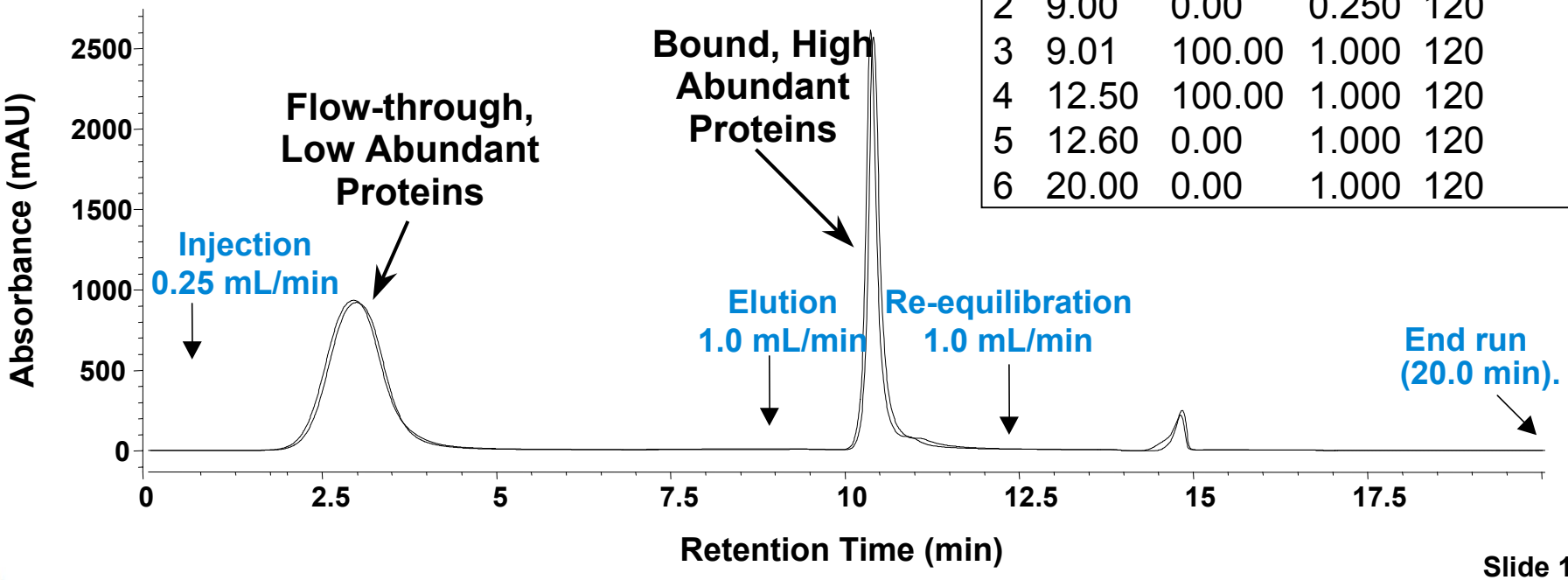
- Total column run cycle = 20.00 min, for injection, elution, and regeneration (4.6 x 50 mm column).
- Multiple Affinity Removal column is reusable, protein binding capacity is unchanged after 200 injections of serum
- Capacity =

  15-20 µL serum per injection

  1.2 - 1.6 mg total serum proteins

	Time (min)	%B	Flow rate	Max. pressure
1	0.00	0.00	0.250	120 bar
2	9.00	0.00	0.250	120
3	9.01	100.00	1.000	120
4	12.50	100.00	1.000	120
5	12.60	0.00	1.000	120
6	20.00	0.00	1.000	120

Comparison of Run #20 and Run #200



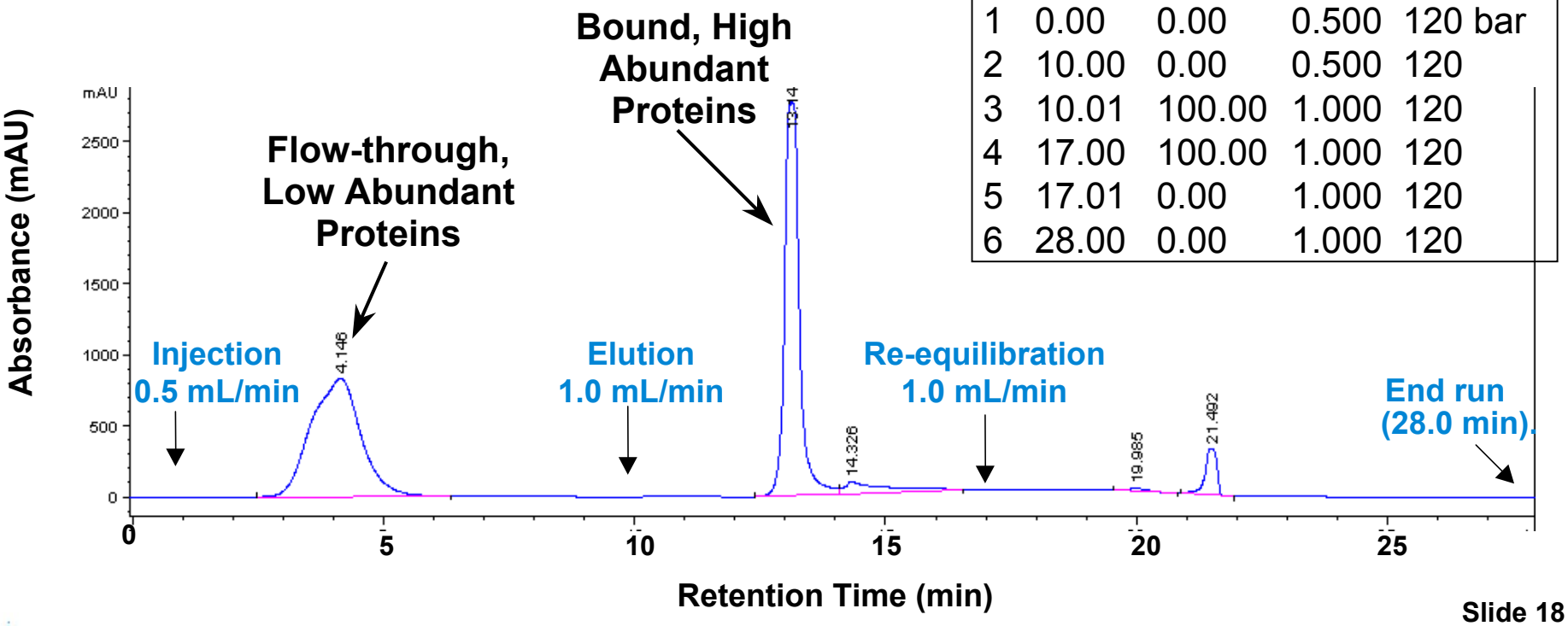
Immunoaffinity Column Elution Profile - 100 mm column

- Total column run cycle = 28.00 min, for injection, elution, and regeneration (4.6 x 100 mm column).
- Capacity =

  30-40 µL serum per injection

  2.4 - 3.2 mg total serum proteins

	Time (min)	%B	Flow rate	Max. pressure
1	0.00	0.00	0.500	120 bar
2	10.00	0.00	0.500	120
3	10.01	100.00	1.000	120
4	17.00	100.00	1.000	120
5	17.01	0.00	1.000	120
6	28.00	0.00	1.000	120



Agilent Technologies

Chairperson: John Vis

How do you use the products?

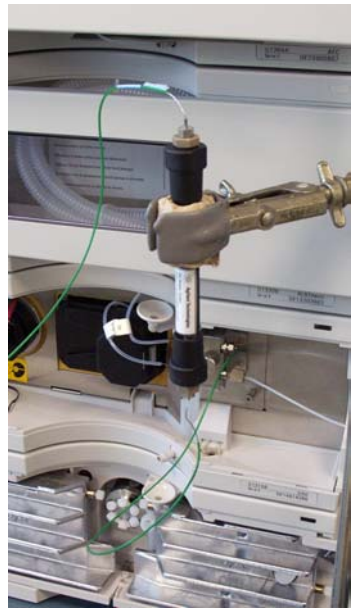
1. Set up Buffers A (column load/wash/equil) and B (elute) and purge lines.

2. Set up LC timetable (100% A, then 100% B, back to 100% A, see-instructions, time and flow rate depends on column size) and run two blanks. Make sure sample loop size is appropriate.

3. Attach column and equilibrate with Buffer A.

4. Dilute human serum 5 x with Buffer A (e.g. 15µL serum plus 60µL Bufffer A) and filter with 0.22um spin filters to remove particulates.

5. Inject diluted sample (consult C of A for true column capacity).



-- More --

How do you use the products? (contd)

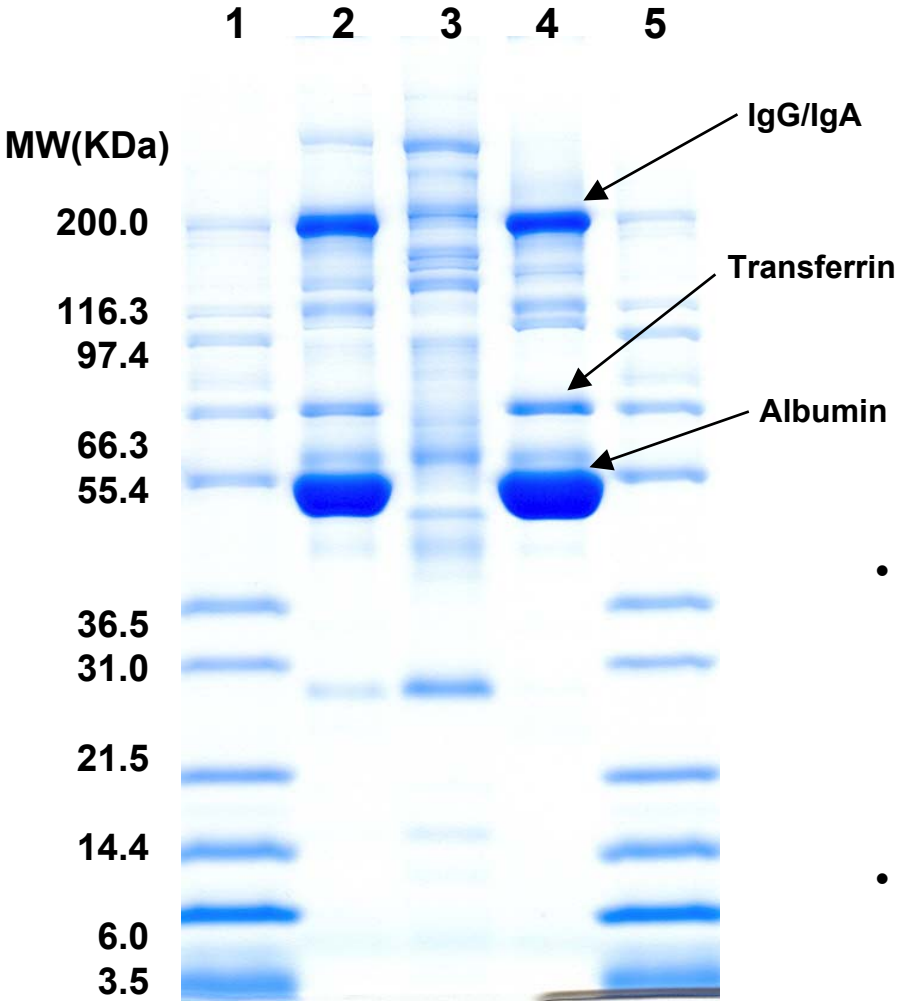
6. Collect flow-through (unretained) fraction = low abundant proteins.

7. Elute bound proteins with Buffer B= high-abundant proteins into waste or save (if they need to be analyzed).

8. Regenerate with Buffer A.

9. Concentrate (and pool if necessary) low-abundant proteins using spin concentrators and analyze the samples.

1D-Gel - Human Serum on Multiple Affinity Removal Column



4-20% SDS PAGE, non-reduced

Lane 1 - Mark12 Standards (Invitrogen)

Lane 2 - Serum, 10ug

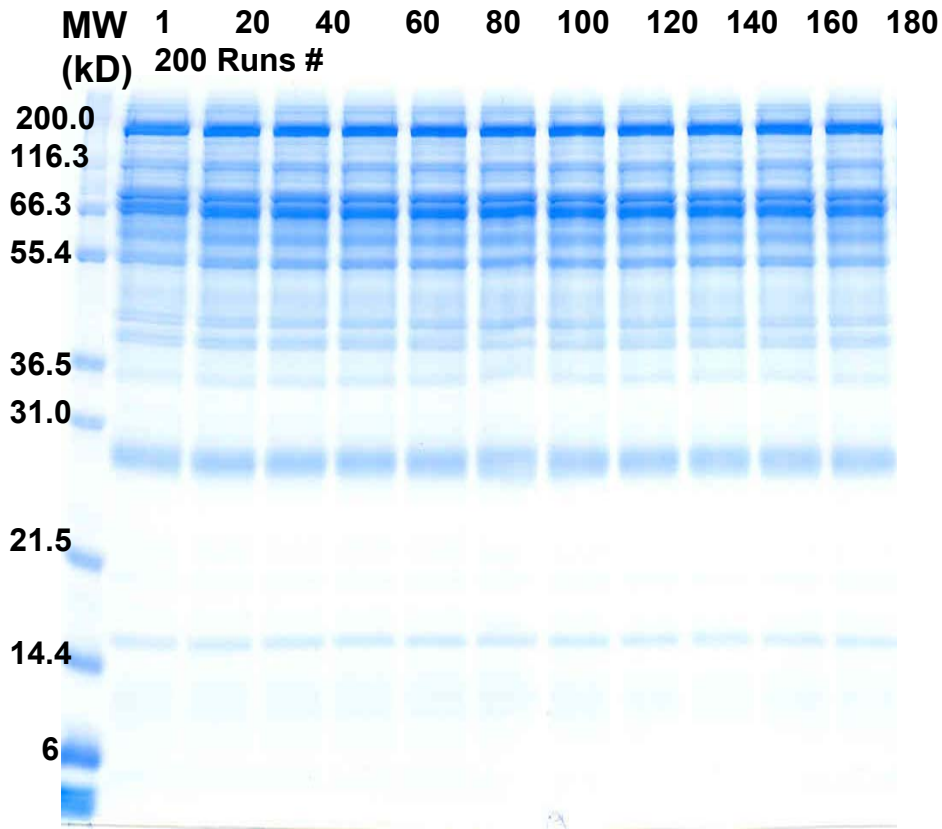
Lane 3 - Flow-through fraction, 10ug

Lane 4 - Bound fraction, 10ug

Lane5 - Mark12 Standards

- **Multiple Affinity Removal column efficiently removes high-abundance proteins from serum. Based on protein assay of the flow-through fraction, more than 85-90% of total protein was removed.**
- **Retained fraction proteins were resolved by SDS-PAGE and analyzed by MALDI and LC/MS. All of the bands were positively identified as target proteins.**

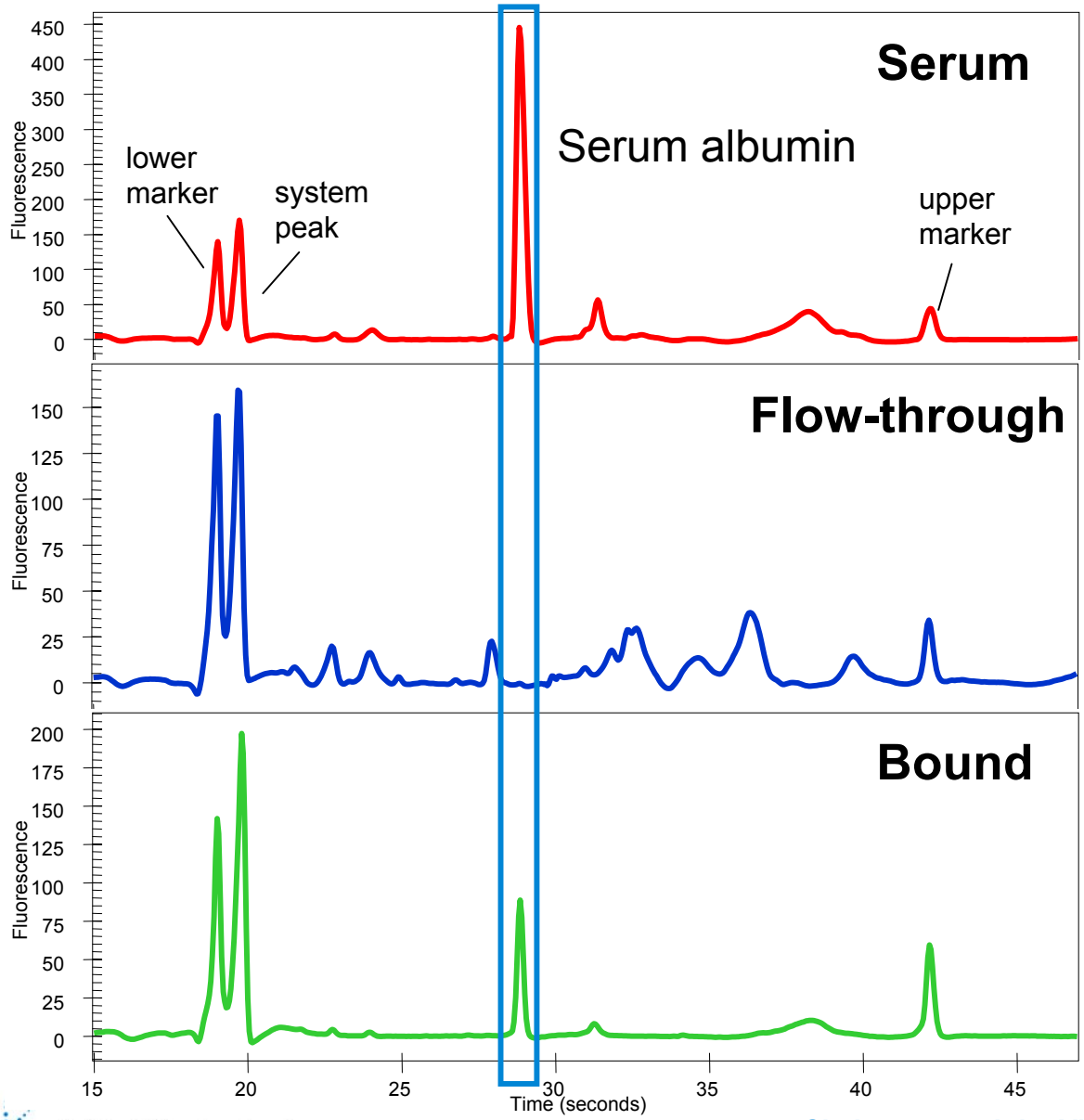
Reproducibility of High Abundance Protein Removal



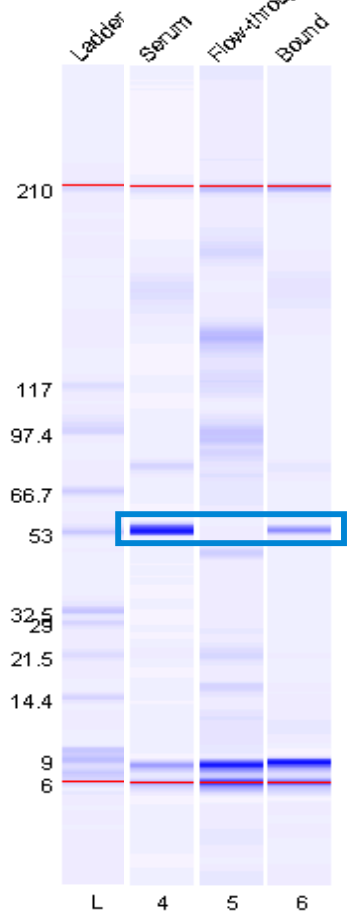
Human Serum

- The correct binding and elution formulations are required for reproducible long life use.
- Reproducible depletion of target proteins from human serum as indicated by constant gel pattern of the depleted serum.
- Protein content of flow-through fractions remains consistent during 200 runs.

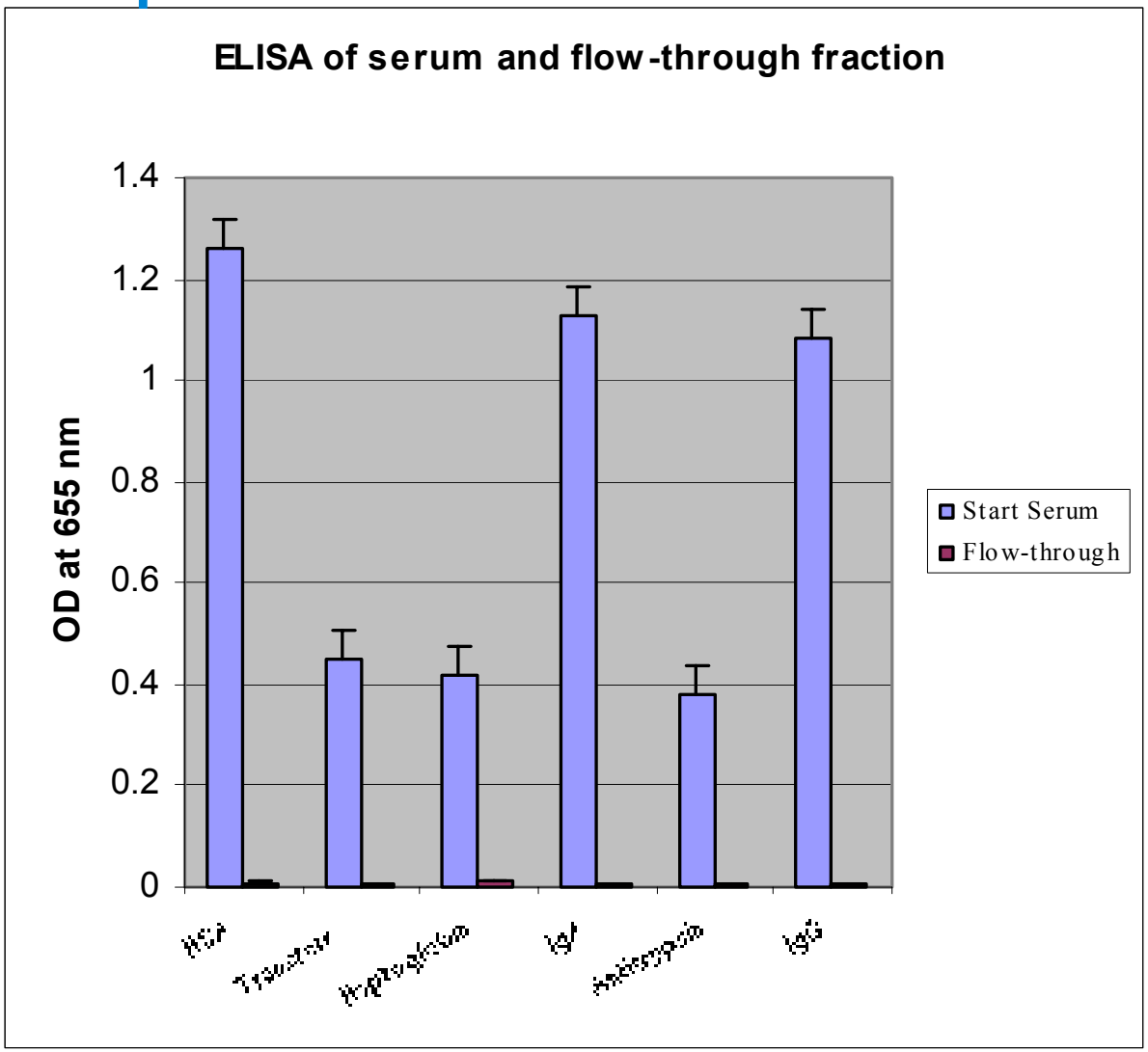
Immunodepletion with Multiple Affinity Removal Column



Bioanalyzer 2100 - provides a quick evaluation of protein content



Removing High Abundance Proteins in Proteomic Samples



More than 98-99+% of targeted high abundance proteins were removed from serum in the single pass immunoaffinity selection.

Selectivity of Immunoaffinity Solid Phase: Binding Analysis

- Solid-phase immuno-selected proteins from normal human serum were resolved on SDS-PAGE and identified bands were cut and processed for ID by tryptic digestion and MALDI/MS or LC/MS(IT).

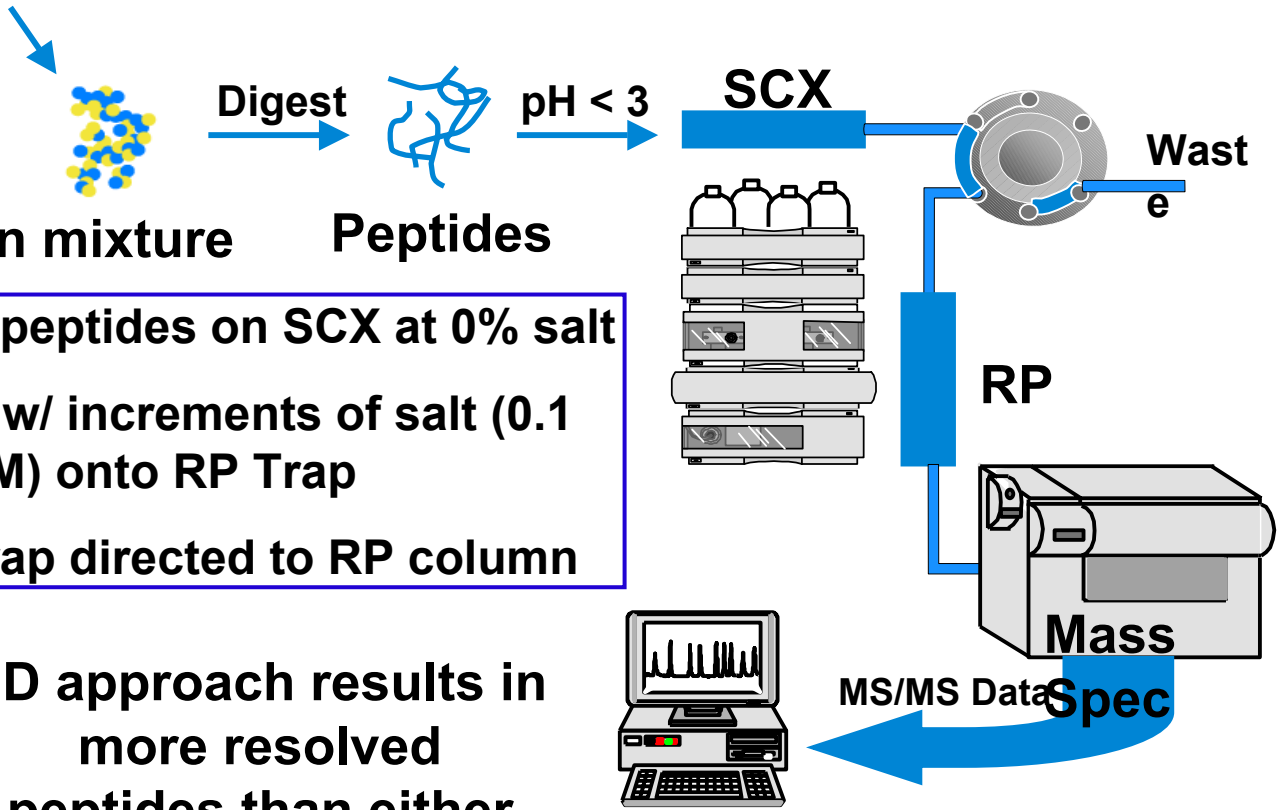
- Proteins in addition to the six targeted proteins in the bound fraction were identified.

- The only other proteins that we observed in very small quantities (not fully captured by the column) were:
  - Complement C3
  - Complement C4
  - Apolipoprotein A-1

- These proteins may be retaining due to association with albumin.

Human Serum Protein Identification by 2D LC/MS (IT): Comparison of IDs based on sample preparation

Serum after depletion by Cibacron Blue or Serum after depletion by Multiple Affinity Removal System



1) Load peptides on SCX at 0% salt
2) Elute w/ increments of salt (0.1 M - 1 M) onto RP Trap
3) RP Trap directed to RP column

2D approach results in more resolved peptides than either single dimension

Data Analysis: Spectrum Mill

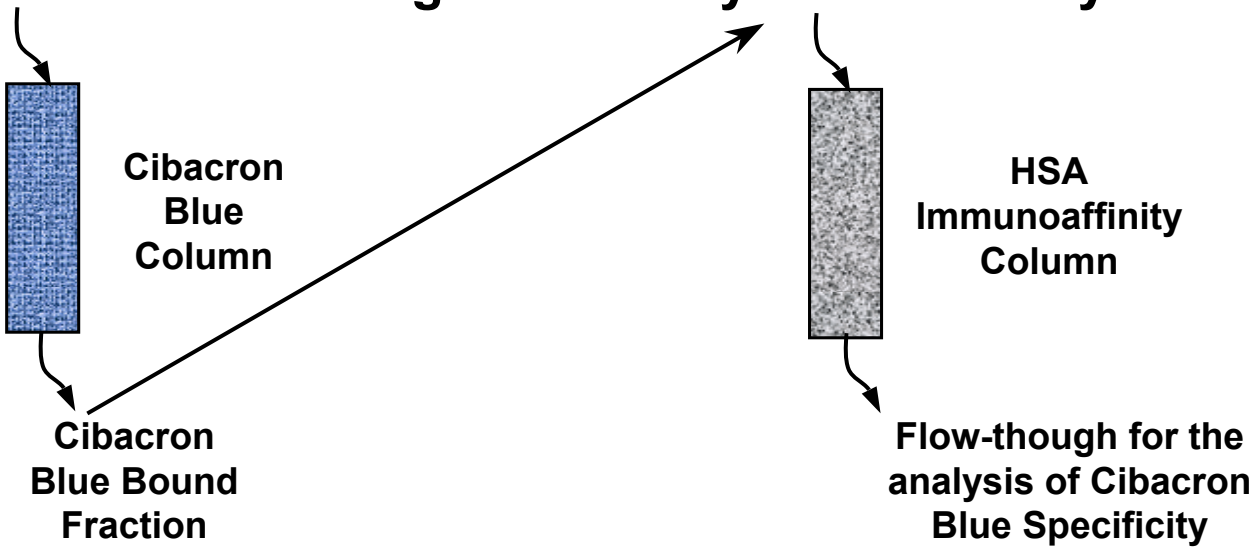
Human Serum Protein Identification by 2D LC/MS (IT):
Comparison of IDs Based on Sample Preparation Method

Cibacron # spectra intensity	Agilent # spectra intensity	Serum # spectra intensity	#	Protein Name
59 5.13e+008	0 0.00e+000	1146 3.06e+010	1	Serum albumin
153 5.76e+009	0 0.00e+000	42 3.32e+008	2	Serotransferrin
58 8.87e+008	98 1.94e+009	37 3.30e+008	3	Alpha-2-macroglobulin
7 4.22e+007	100 1.06e+009	26 1.07e+008	4	Complement C3
109 4.36e+009	14 1.67e+008	34 7.70e+008	5	Alpha-1-antitrypsin
28 6.52e+008	26 3.57e+008	9 4.76e+007	6	Vitamin D-binding protein
30 1.88e+009	0 0.00e+000	16 2.35e+008	7	Haptoglobin
0 0.00e+000	87 5.55e+008	19 2.94e+008	8	complement C4
78 4.43e+009	1 2.59e+006	34 1.49e+009	9	IgG1
21 2.09e+008	68 1.30e+009	28 2.53e+008	10	Apolipoprotein A-I
69 1.01e+009	5 4.02e+007	12 1.76e+008	11	Ig alpha
0 0.00e+000	24 1.81e+008	7 2.45e+007	12	hemopexin
25 1.72e+009	2 2.57e+007	12 3.65e+008	13	immunoglobulin kappa
0 0.00e+000	17 8.19e+007	6 8.79e+006	14	Fibronectin
50 7.36e+008	57 5.27e+008	18 1.15e+008	15	Transthyretin
0 0.00e+000	11 1.27e+008	3 2.03e+006	16	ITIH1
0 0.00e+000	28 9.32e+007	5 1.69e+007	17	ITIH2
0 0.00e+000	12 1.24e+008	0 0.00e+000	18	ITIH4
9 4.45e+007	3 4.16e+007	10 5.04e+007	19	Ig mu
19 3.52e+008	14 7.47e+007	7 4.85e+007	20	α-2-HS glycoprotein
0 0.00e+000	32 2.50e+008	8 1.23e+008	21	B-factor, properdin
9 1.21e+008	12 6.93e+007	3 6.76e+006	22	Prothrombin
6 4.70e+007	74 1.16e+009	10 1.26e+008	23	Apolipoprotein A-II
1 3.03e+006	11 9.66e+007	2 8.40e+006	24	Cerulopdlasmin
19 1.66e+009	5 1.19e+008	25 2.09e+009	25	Ig lambda
8 4.63e+007	0 0.00e+000	0 0.00e+000	26	Zn-α-2-glycoprotein
14 8.80e+007	8 1.19e+008	1 5.22e+006	27	orosomucoid 1
0 0.00e+000	22 1.36e+008	1 8.35e+006	28	alpha-1-antichymotrypsin
0 0.00e+000	8 5.92e+007	1 1.52e+006	29	Plasma protease C1 inhibitor
0 0.00e+000	8 2.60e+007	4 7.25e+006	30	Complement factor H
8 9.03e+007	8 1.03e+008	4 4.10e+007	31	alpha 1B-glycoprotein
0 0.00e+000	4 1.55e+007	2 1.31e+006	32	Kininogen, LMW precursor
0 0.00e+000	4 2.58e+007	6 3.78e+007	33	Apolipoprotein B-100
3 2.18e+007	0 0.00e+000	0 0.00e+000	34	Lumican
0 0.00e+000	4 5.77e+007	3 9.48e+006	35	Clusterin
0 0.00e+000	3 5.74e+007	2 4.35e+007	36	apolipoprotein H
4 5.23e+007	1 1.72e+007	0 0.00e+000	37	Leucine-rich α-2-glycoprotein
5 6.00e+007	1 2.85e+006	4 8.83e+006	38	Trypsin
0 0.00e+000	8 3.35e+007	4 1.30e+007	39	Complement C5
2 4.45e+007	0 0.00e+000	1 2.61e+007	40	Ig Kappa
2 5.90e+006	1 1.30e+007	0 0.00e+000	41	angiotensinogen
2 3.04e+006	7 3.46e+007	0 0.00e+000	42	Antithrombin-III
0 0.00e+000	3 6.39e+006	2 4.78e+006	43	Carboxypeptidase
0 0.00e+000	1 1.03e+006	4 7.28e+006	44	Platelet factor 4
0 0.00e+000	1 5.46e+006	1 1.83e+006	45	plasmin
4 1.12e+007	0 0.00e+000	0 0.00e+000	46	beta globin chain variant
1 1.75e+007	0 0.00e+000	1 1.06e+007	47	Ig kappa chain V-I region

Chairperson: John Vis

Selectivity of Cibacron Blue Solid Phase: Binding Analysis

Analysis of CB-Resin Binding Proteins by Serial Affinity Columns



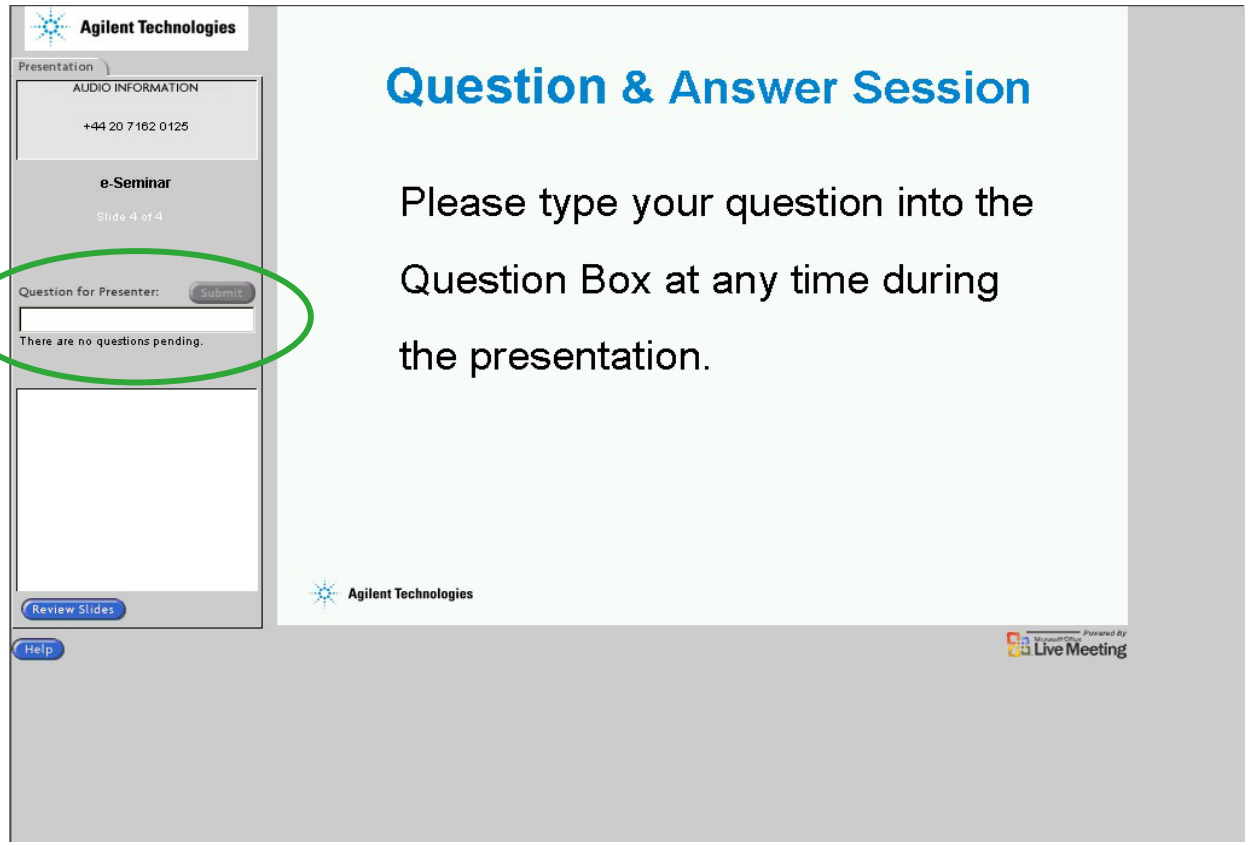
A. Selection by CB-Resin

1. Sample in Binding Solution: phosphate buffer, low salt
2. Wash with Binding Solution
3. Collect Sample after Desorption: phosphate buffer, 1.5 M KCl

B. HSA Removal by Immunoaffinity Column

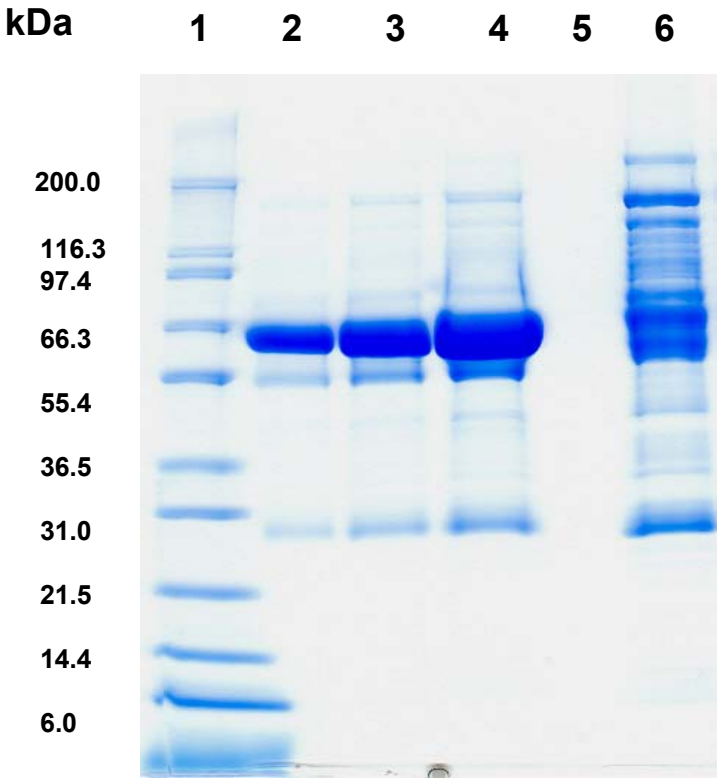
1. Dilute High Salt CB-Elute 1:1 with phosphate buffer
2. Collect Flow-Through for Analysis

Break Number 2



Selectivity of Cibacron Blue Solid Phase: Binding Analysis

A Lot of Proteins Bind to CB besides HSA!



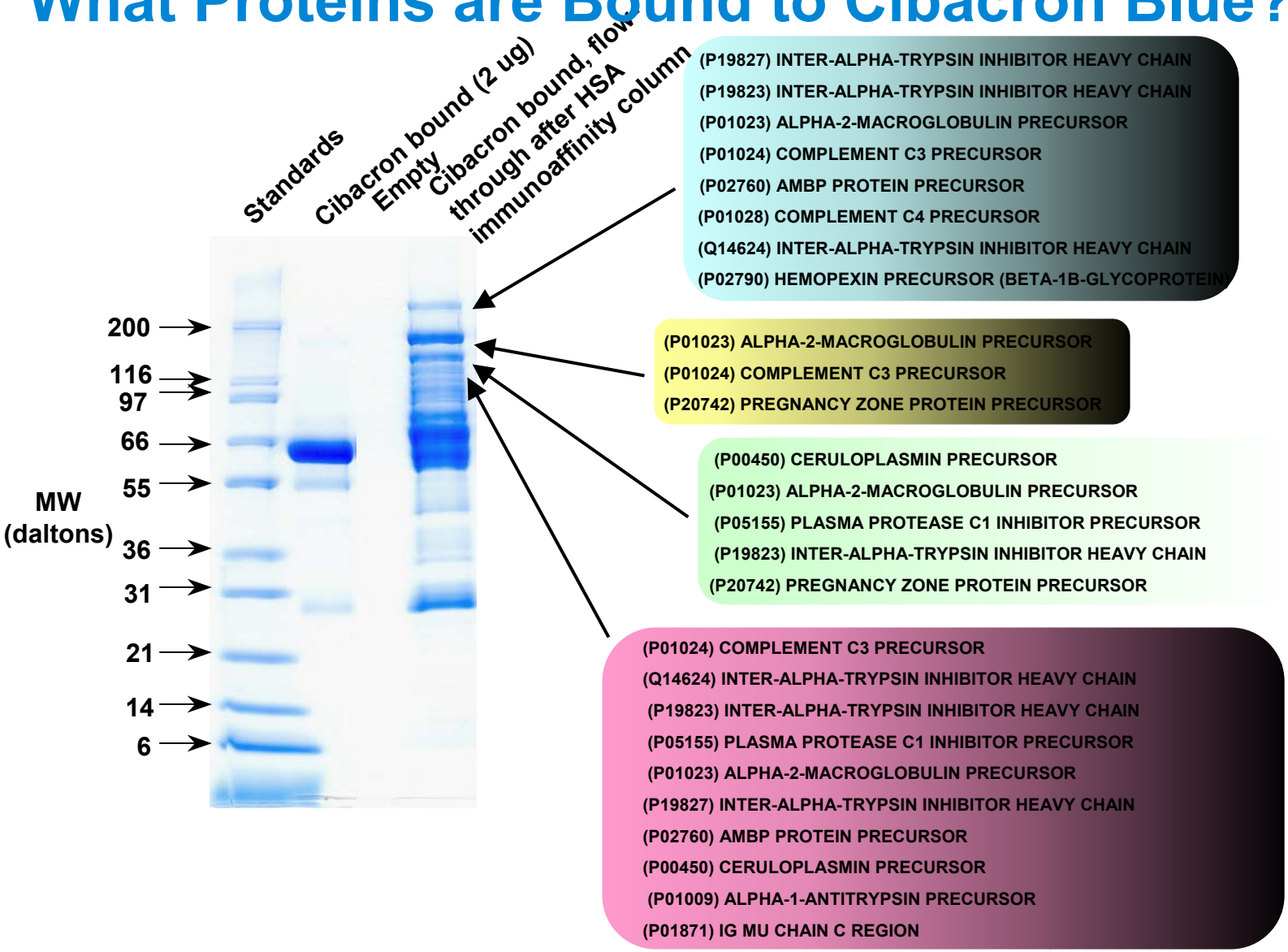
#1 - Mark12 standards
#2 - Cibacron bound, 2ug
#3 - Cibacron bound, 4ug
#4 - Cibacron bound, 6ug
#5 - empty
#6 - Cibacron bound, Flow-through after HSA affinity column

4-20% SDS PAGE (non-reducing)

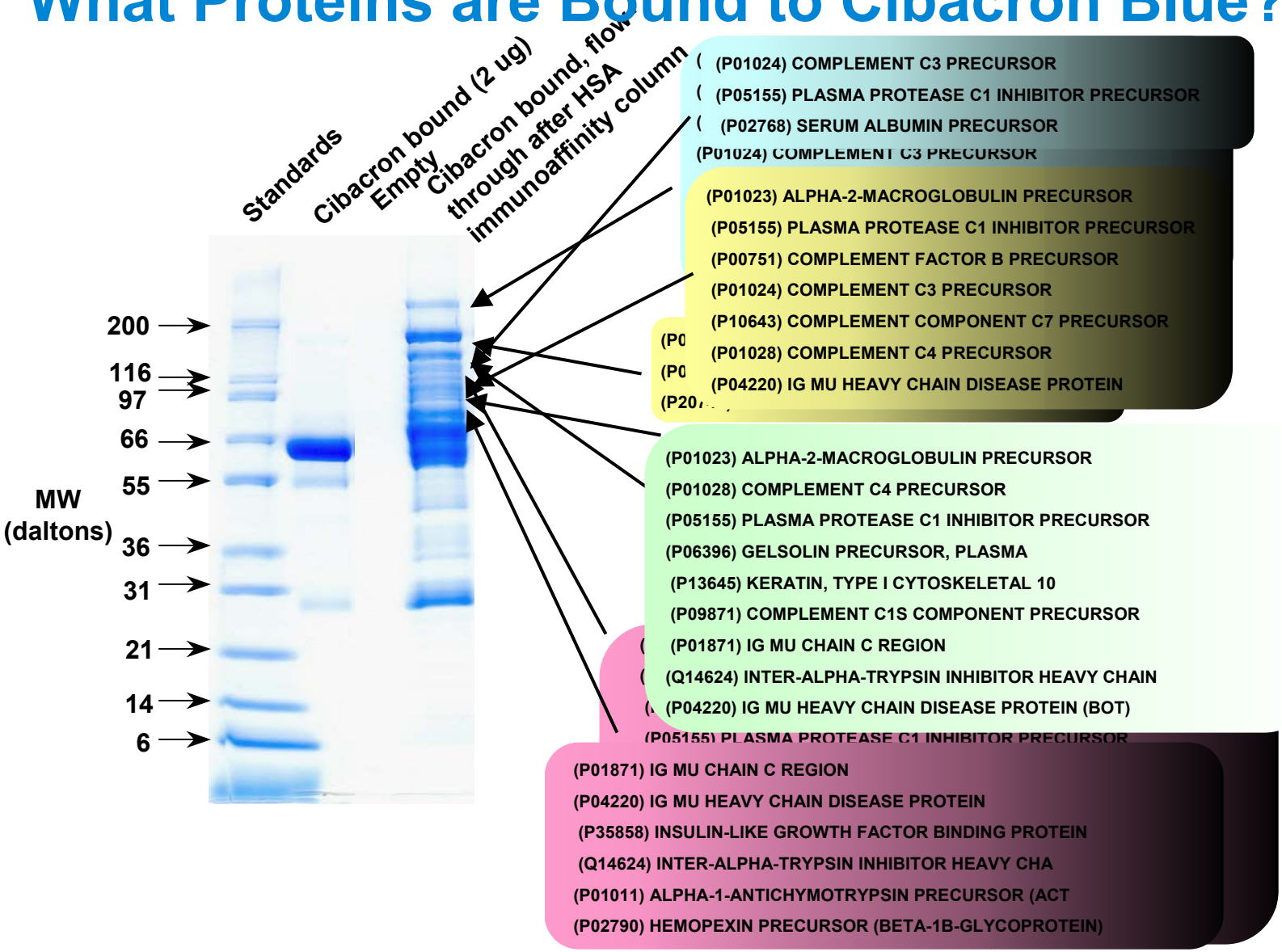
Agilent Technologies

Chairperson: John Vis

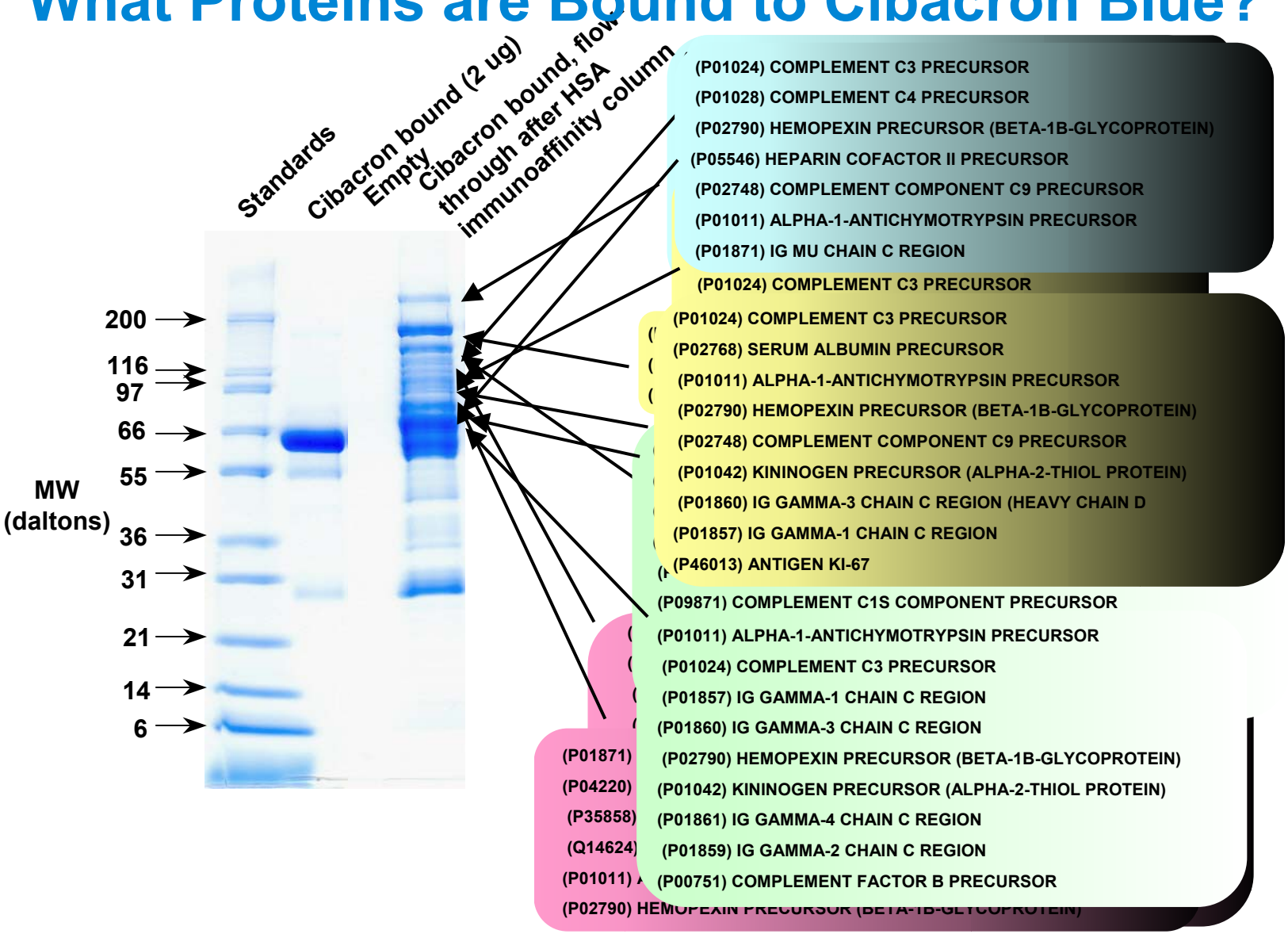
What Proteins are Bound to Cibacron Blue?



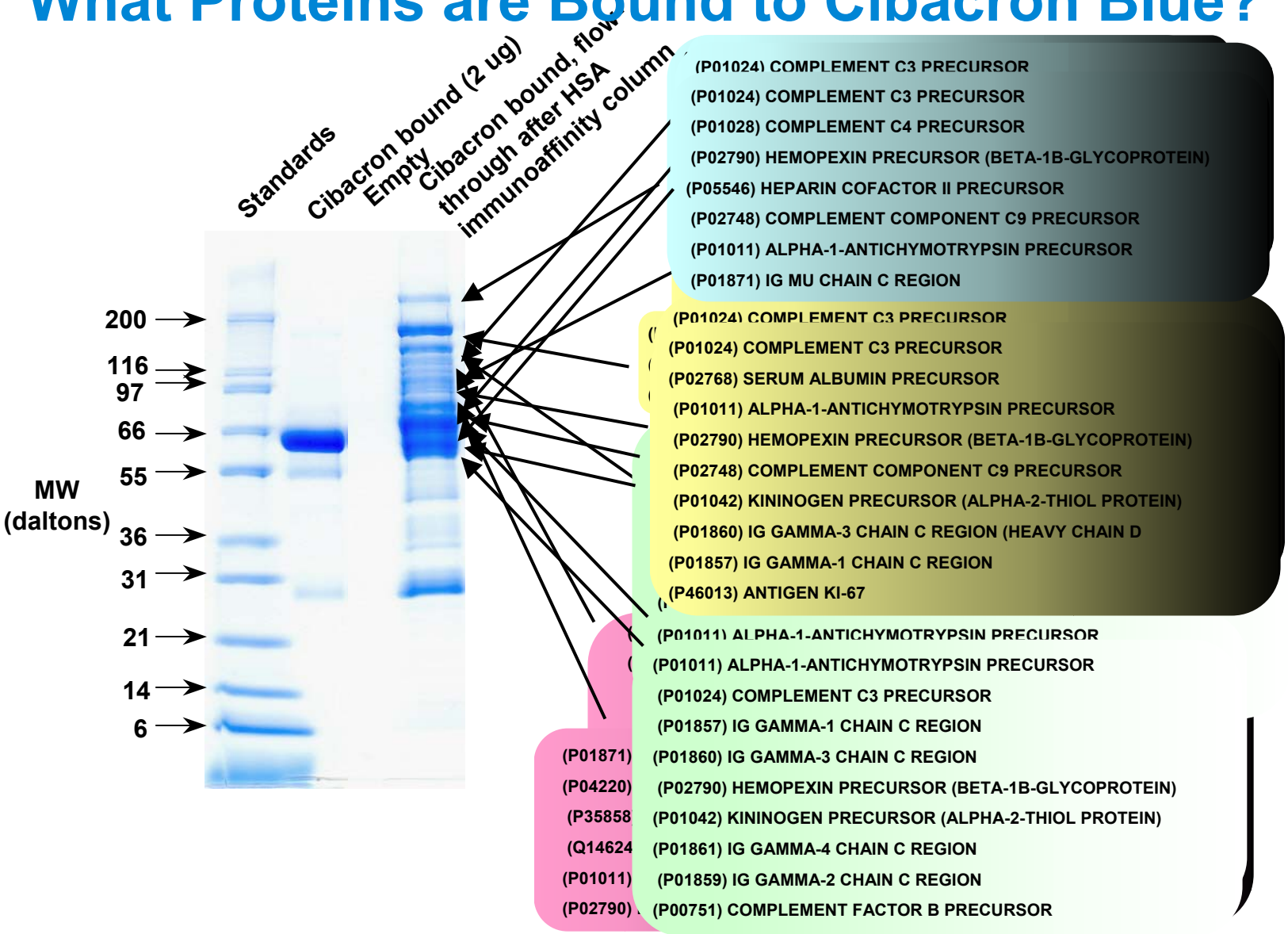
What Proteins are Bound to Cibacron Blue?



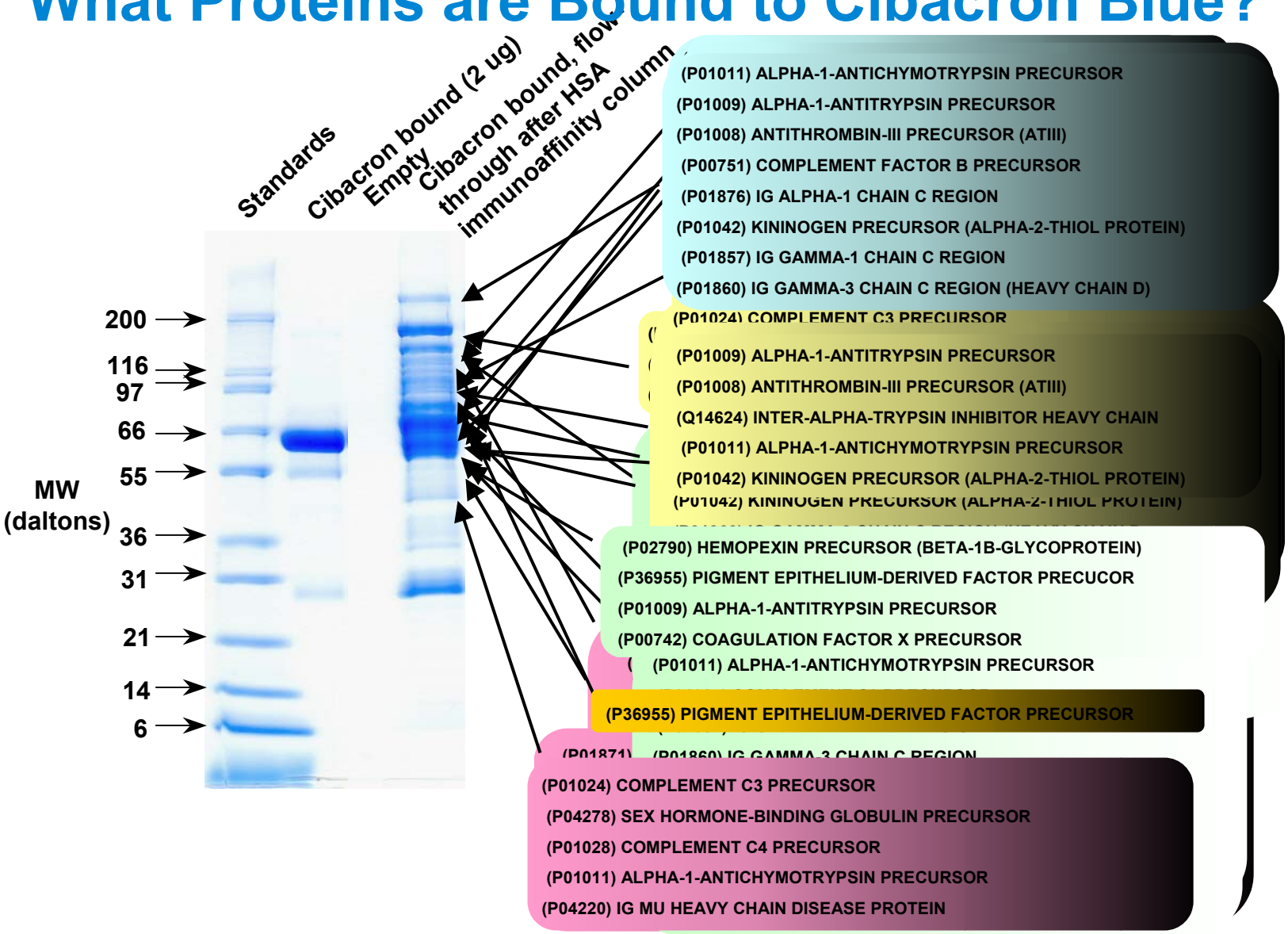
What Proteins are Bound to Cibacron Blue?



What Proteins are Bound to Cibacron Blue?



What Proteins are Bound to Cibacron Blue?



1	A1AT_HUMAN	(P01009) ALPHA-1-ANTITRYPSIN PRECURSOR
2	A2MG_HUMAN	(P01023) ALPHA-2-MACROGLOBULIN PRECURSOR
3	AACT_HUMAN	(P01011) ALPHA-1-ANTICHYMOTRYPSIN PRECURSOR
4	ALBU_HUMAN	(P02768) SERUM ALBUMIN PRECURSOR
5	ALC1_HUMAN	(P01876) IG ALPHA-1 CHAIN C REGION
6	ALS_HUMAN	(P35858) INSULIN-LIKE GROWTH FACTOR BINDING PROTE
7	AMBP_HUMAN	(P02760) AMBP PROTEIN PRECURSOR
8	ANT3_HUMAN	(P01008) ANTITHROMBIN-III PRECURSOR (ATIII)
9	APA1_HUMAN	(P02647) APOLIPOPROTEIN A-I PRECURSOR (APO-AI)
10	C1S_HUMAN	(P09871) COMPLEMENT C1S COMPONENT PRECURSOR
11	CERU_HUMAN	(P00450) CERULOPLASMIN PRECURSOR (EC 1.16.3.1)
12	CFAB_HUMAN	(P00751) COMPLEMENT FACTOR B PRECURSOR (EC 3.4.2)
13	CLUS_HUMAN	(P10909) CLUSTERIN PRECURSOR
14	CO3_HUMAN	(P01024) COMPLEMENT C3 PRECURSOR
15	CO4_HUMAN	(P01028) COMPLEMENT C4 PRECURSOR
16	CO7_HUMAN	(P10643) COMPLEMENT COMPONENT C7 PRECURSOR
17	CO9_HUMAN	(P02748) COMPLEMENT COMPONENT C9 PRECURSOR
18	FA10_HUMAN	(P00742) COAGULATION FACTOR X PRECURSOR
19	GC1_HUMAN	(P01857) IG GAMMA-1 CHAIN C REGION
20	GC2_HUMAN	(P01859) IG GAMMA-2 CHAIN C REGION
21	GC3_HUMAN	(P01860) IG GAMMA-3 CHAIN C REGION
22	GC4_HUMAN	(P01861) IG GAMMA-4 CHAIN C REGION
23	GELS_HUMAN	(P06396) GELSOLIN PRECURSOR
24	HEMO_HUMAN	(P02790) HEMOPEXIN PRECURSOR
25	HEP2_HUMAN	(P05546) HEPARIN COFACTOR II PRECURSOR (HC-II)
26	HPTR_HUMAN	(P00739) HAPTOGLOBIN-RELATED PROTEIN PRECURSOR
27	IC1_HUMAN	(P05155) PLASMA PROTEASE C1 INHIBITOR PRECURSOR
28	ITH1_HUMAN	(P19827) INTER-ALPHA-TRYPSIN INHIBITOR
29	ITH2_HUMAN	(P19823) INTER-ALPHA-TRYPSIN INHIBITOR
30	ITH4_HUMAN	(Q14624) INTER-ALPHA-TRYPSIN INHIBITOR
31	KAC_HUMAN	(P01834) IG KAPPA CHAIN C REGION
32	KI67_HUMAN	(P46013) ANTIGEN KI-67
33	KNG_HUMAN	(P01042) KININOGEN PRECURSOR
34	KV2C_HUMAN	(P01616) IG KAPPA CHAIN V-II REGION
35	KV2F_HUMAN	(P06310) IG KAPPA CHAIN V-II REGION
36	KV3B_HUMAN	(P01620) IG KAPPA CHAIN V-III REGION
37	KV3E_HUMAN	(P01623) IG KAPPA CHAIN V-III REGION
38	LAC_HUMAN	(P01842) IG LAMBDA CHAIN C REGIONS
39	LV1B_HUMAN	(P01700) IG LAMBDA CHAIN V-I REGION
40	LV1F_HUMAN	(P04208) IG LAMBDA CHAIN V-I REGION
41	MUC_HUMAN	(P01871) IG MU CHAIN C REGION
42	MUCB_HUMAN	(P04220) IG MU HEAVY CHAIN DISEASE PROTEIN
43	PEDF_HUMAN	(P36955) PIGMENT EPITHELIUM-DERIVED FACTOR PRECURSOR
44	PZP_HUMAN	(P20742) PREGNANCY ZONE PROTEIN PRECURSOR
45	SAMP_HUMAN	(P02743) SERUM AMYLOID P-COMPONENT PRECURSOR
46	SHBG_HUMAN	(P04278) SEX HORMONE-BINDING GLOBULIN PRECURSOR

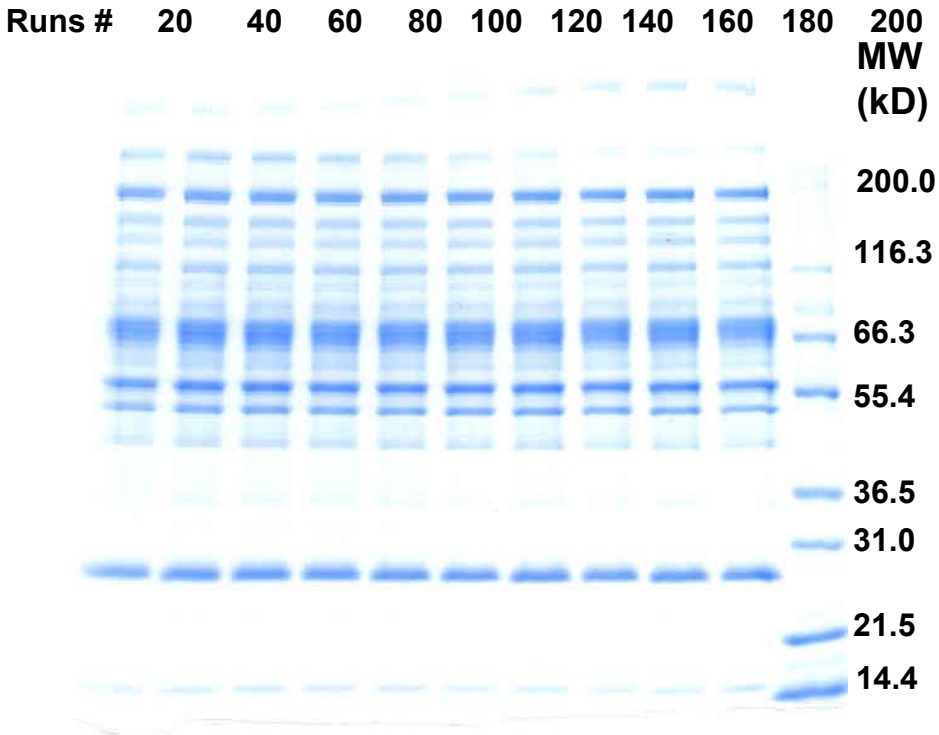
Proteins Bound to Cibacron Blue: SDS-PAGE, 1D-LC/MS(IT)

Serum Proteins Bound to Cibacron Blue: 2D-LC/MS(IT)

(#)	Protein Name
1	Alpha-2-macroglobulin
2	Complement C3
3	complement C4
4	Ceruloplasmin
5	Alpha-1-antitrypsin
6	Serum albumin precursor
7	α-1-antitrypsin
8	Apolipoprotein A-I
9	keratin 1
10	Antithrombin-III
11	ITIH2
12	ITIH1
13	C1 inhibitor
14	ITIH4
15	hemopexin
16	complement factor B
17	kininogen, LMW
18	Ig mu chain
19	Gelsolin
20	Igkappa light chain
21	Heparin cofactor II
22	Serum amyloid P-component
23	Trypsin precursor (pig)
24	Complement component C7
25	complement 9
26	Ig heavy chain
27	alpha-2 antiplasmin
28	Clusterin
29	Ig alpha-1 chain C region
30	peptidoglycan recognition protein L
31	keratin 10
32	insulin-like growth factor binding protein
33	Ig lambda light chain VLJ region
34	haptoglobin-related protein
35	α-1 microglycoprotein
36	Afamin precursor
37	Ig heavy chain variable region
38	trypsinogen hL
39	Ig alpha heavy chain variable region
40	Ig kappa light chain VLJ region
41	cytokeratin 9
42	trypsinogen 16
43	complement C6
44	ATP-binding
45	angiotensin
47	Complement C5
48	KIAA1461 protein
52	Ig lambda light chain variable region
53	ATP synthase F0 subunit 6
54	Ig heavy chain V-III region HIL
55	hypothetical protein XP_289343
56	S-protein precursor
57	embryonic leucine zipper kinase
59	cul-3
61	unnamed protein product
62	Coagulation factor X precursor (Stuart factor)
65	Serum aryldiakylphosphatase 1
66	Complement C1s
72	TFNR
76	glycosylphosphatidylinositol phospholipase D
78	KIAA1926 protein
79	Plasminogen

Multiple Affinity Removal System for Plasma - 1DGE Results

Human Plasma



- In addition to serum, Multiple Affinity Removal System works for human plasma.
- No EDTA was needed to prevent coagulation.
- No column plugging was observed over 200 injections onto a column.
- Independent tests with Cerebrospinal Fluid indicate similar results.
- Urine samples are of potential
- Tissues contaminated with blood

Can the Human Multiple Affinity Removal Columns be used with other species?

- We have tested the human antibody columns with mouse, rat, bovine, sheep, and other serum proteins.
- Very little binding of high-abundant proteins was found for other species.
- Columns are only recommended for proteins from human biological fluids.

Can the Human Multiple Affinity Removal Columns be used with other species?

- We have tested the human antibody columns with mouse, rat, bovine, sheep, and other serum proteins.
- Very little binding of high-abundant proteins was found for other species.
- Columns are only recommended for proteins from human biological fluids.

Can the Human Multiple Affinity Removal Columns be used with other species?

- We have tested the human antibody columns with mouse, rat, bovine, sheep, and other serum proteins.
- Very little binding of high-abundant proteins was found for other species.
- Columns are only recommended for proteins from human biological fluids.

- But stay tuned….

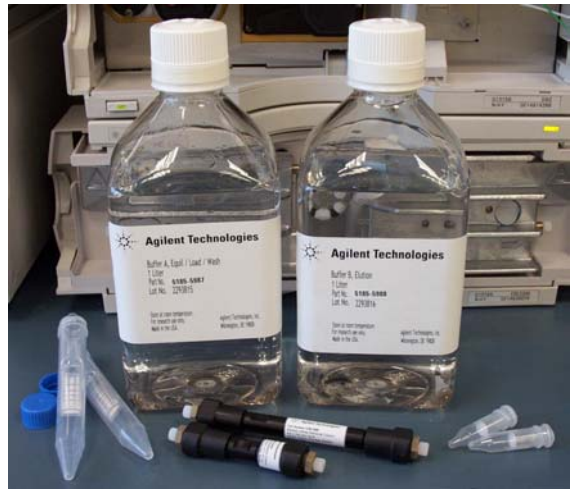
What do I do if I need more protein mass for analysis, or to lyophilize my proteins?

- Although the Multiple Affinity Removal Columns have capacity for 15-20uL or 30-40 uL of serum per injection, collected fractions can be pooled and concentrated for analysis.
- Spin concentrators can be used (supplied by Agilent), or any protein concentration technique.
- Collected fractions are diluted in Buffer A, which contains <0.02% sodium azide as a preservative.
- If lyophilizing the collected proteins is desired, a buffer exchange is recommended after concentrating, to a more volatile buffer (e.g. ammonium bicarbonate).

Product availability

- Two stock columns sizes: 4.6 x 50mm and 4.6 x 100mm.
- Custom column sizes will be considered.
- Reagent Kit with buffers, spin filters and spin concentrators is available.
- The buffers must be used with the columns to maximize column lifetime.
- All kit contents are orderable individually.
- Available now for ordering.

The Starter Reagent Kit contains:



- 2 bottles Buffer A
- 1 bottle Buffer B
- 2 packs of 25 Spin Filters
- 1 pack of 25 Spin Concentrators

- Kit should last (under normal usage conditions) for the 200 injection lifetime of a 4.6 x 50mm column; for half the life (100 injections) of a 4.6 x 100mm column.
- Monitor reagent usage and replenish as necessary.
- An HSA standard will be available to use to check column performance periodically (not in kit).

Conclusions

Agilent Multiple affinity removal system

- Selectivity – a small number of untargeted proteins are removed.
- Capacity – predictable and stable
  - 4.6 x 50 mm (0.8 mL) = 15-20 uL of serum
  - 4.6 x 100 mm (1.6 mL) = 30-40 uL of serum.
- Cross Contamination – no proteins apparent in “blanks”; no loss of capacity.
- Simple and Fast Use Conditions – two buffers, simple LC, less than 30 minute/sample.
- Compatible With Purpose – after sample concentration, compatible with one and two dimensional PAGE, LC, and/or digestion methods.
- Tailored to Sample – tested on human serum, plasma, and CSF.
- Enabling - expands dynamic range of 1DGE, 2DGE, LC/MS, and Bioanalyzer 2100 for biomarker identification.

Summary

- New product for removing SIX HUMAN serum proteins (from serum, plasma and other fluids too).
- LC-format columns, reagents, spin filters, spin concentrators, and a starter kit.
- For more details see www.agilent.com/chem/affinity.