

# Separation techniques (Proteins)

## Affinity Chromatography

### Purification of antibodies : IgG binding protein supports

#### Protein A, G, L - Affaroses

Antibody purification was popularized by Protein A affinity chromatography. Uptima offers high-quality supports with Ig-binding proteins, rProtein A, rProtein G, rProtein L for the requirements from R&D level to production (ask for bulk sizes).

Choose the right support considering :

- ◆ the specificity of the ligands,
- ◆ other parameters such as stability, leaching, flow rate...

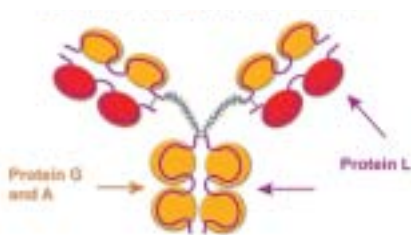
Protein A suits particularly the purification of monoclonal antibodies from FCS supplemented cell cultures. It is often the first intention of choice for many other Ig species, but Protein G may be preferred when cross-reactivity to several species is desired, or when Protein A fails. Protein L is dedicated to specific applications especially Ig fragments purification.

Protein A, Protein G and Protein L are available in recombinant grade, with unsurpassed binding capacity.

**Affarose format** (bulk resins for Protein A and L) : Ligands are covalently bound to the matrice, a modified agarose called Affarose, to ensure minimal leaching of ligand, excellent stability and to allow re-use.

**UptiSpin format** (ready-to-use kit for Protein A and G) : Designed for simple, rapid and multiple antibody purification from serum, ascites and tissue culture supernatant. Also allow re-use.

Binding Sites for protein G, A and L



(native)	Protein A (Staphylococcus)	Protein G (Streptococcus)	Protein L (Peptostreptococcus)
Origin	E. coli	E. coli	E. coli
Nb binding sites	(4) 4	(4) 2	4
Ig binding site	Fc	Fc	K light chain
Albumin binding	(-) -	(+) -	+
Optimal pH binding	8-9	5-6	7.5

#### Binding capacity \*

Hu IgGs	+++	+++	+++
Hu IgG1	+++	+++	+++
Hu IgG2	+++	+++	+++
Hu IgG3	+	+++	+++
Hu IgG4	+++	+++	+++
Hu IgM	+	-	+++
Hu IgE	++	-	+++
Hu IgD	-	-	+++
Hu IgA	+	-	+++
Ms IgGs	+++	+++	+++
Ms IgG1	+	++	+++
Ms IgG2a	+++	+++	+++
Ms IgG2b	+++	+++	+++
Ms IgG2c	+++	+++	+++
Ms IgG3	+++	+++	+++
Ms IgM	-	-	++
Rt IgGs	+	++	+++
Rt IgG1	+	++	++
Rt IgG2a	+++	+++	+++
Rt IgG2b	+++	+++	+++
Rt IgG3	+++	+++	+++
Rb IgGs	+++	+++	+
Gp IgGs	+++	+	+/-
Hs IgGs	++		+/-
Dog IgGs	+++	+	
Cat IgGs	+++	+	
Mk IgGs	+++	+++	
Pig IgGs	+++	+	+++
Gt IgGs	+/-	+++	-
Sh IgGs	+/-	+++	+
Bv IgGs	+/-	+++	+/-
Dk IgGs	++	+++	
Hs IgGs	+	+++	
Ck IgGs	-	-	-

Bv : Bovine    Ck : Chicken    Ct : Cat  
 Dg : Dog    Gp : Guinea-pig    Gt : Goat  
 Hm : Hamster    Hs : Horse    Hu : Human  
 Mk : Monkey    Ms : Mouse    Rt : Rat  
 Rb : Rabbit    Sh : Sheep

\* binding capacities for proteins L are given for K subclass only

+++ : strong binding  
 - : no binding

### Affarose rProtein A, XTrem

*The highest capacity support !*

Protein A immobilized onto a 6% cross-linked agarose, supplied at 50% suspension

- ◆ Capacity : 30-40 mg of human IgG per ml of wet gel (static)
- ◆ Excellent stability and minimal leaching of ligand
- ◆ High flow rate, up to 400 cm/hour
- ◆ Pharmaceutical requirements

Applications :

- ◆ Purification of monoclonal antibodies, notably from FCS supplemented culture media (no Bovine IgGs binding)
- ◆ Purification of polyclonal antibodies
- ◆ Immunoprecipitation
- ◆ IgG immobilization (affinity columns preparation)
- ◆ Preadsorption, IgG depletion
- ◆ Isolation of immune complexes

Cycle	Capacity (% initial)	Protein A in eluat
1	100%	2.5 ng/ml*
2	100%	2.4 ng/ml*
16	100%	1.5 ng/ml*
32	>95%	6.7 ng/ml*
100	>85%	3.3 ng/ml*

\*representative datas.

Eluat: polyclonal human IgG 7.4-8.5mg/ml

Product	Cat.#	Qty
Affarose rProtein A, XTrem	UP904670	5 ml
Affarose rProtein A, XTrem	UP904672	20 ml

### Affarose rProtein A

*The classic and perfect choice for most applications*

Protein A immobilised onto a 6% modified agarose, supplied at 50% suspension

- ◆ Lowest leaching (<3ng Protein /mL)
- ◆ Capacity : 18-22 mg of human IgG per ml of wet gel
- ◆ High flow rate, up to 300cm/hour

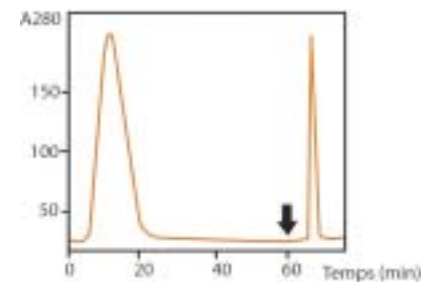
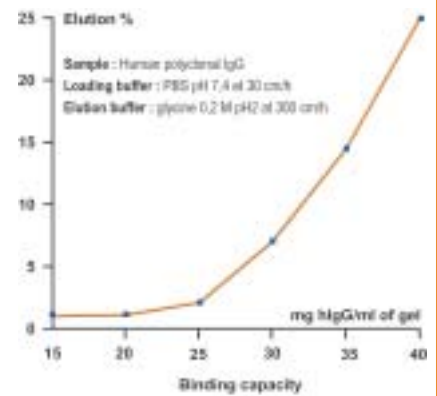
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- ◆ Isolation of immune complexes

Product	Cat.#	Qty
Affarose rProtein A	UP49981G	2 ml
Affarose rProtein A	UP49981H	5 ml

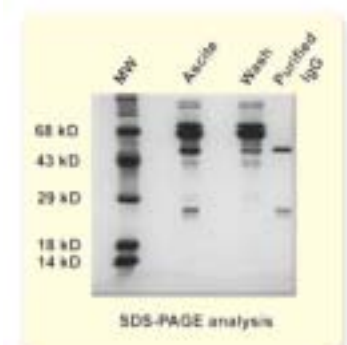
### Technical tip

Protein A binds human IgG subclasses, IgM (medium), IgA and IgE, and mouse IgG1 (weakly), IgG2a and IgG2b. Protein A also binds IgGs from various other laboratory and domestic animals (+++/Rb, Mo, Pg, Dg, Ct, Hu, Gp, ++/Dk), but predominantly only isotypes from some animals (IgG2/Sh, Bv, Gt, IgG2c/Rt).



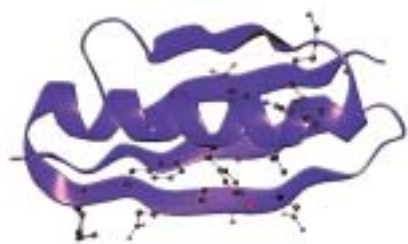
Monoclonal IgG1 purification from an ascite on affarose rProteinA (UP499816).

Uptima Protein A (cat. # UP275757, 10mg) is produced by recombinant technology in E.coli, with culture media being exempt of any animal proteins. Highly controlled procedures give a material free from toxic bacterial contaminant (Staphylococcus endotoxins and hemolysin), found normally in native Protein A.



# Separation techniques (Proteins)

## Affinity Chromatography



3D structure of one sub-unit of rProtein L.  
Drs T. Wan, B.J. Sutton et m. G. Gore,  
university of Southampton, GB.

### Technical tip

Protein L is a protein receptor from *Peptostreptococcus magnus* that binds to kappa light chains without interfering with antigen binding site.

Like Protein A and Protein G, one Protein L molecule contains 4 binding sites. Optimal binding occurs at pH 7.5.

### Affarose rProtein L

Great for the purification of Ig fragments !

Protein L immobilised onto a 4% modified agarose, supplied at 50% suspension

- ◆ Capacity : 3-5 mg of human IgG per ml of wet gel
- ◆ Ig binding does not interfere with antigen binding site
- ◆ Excellent stability

Applications :

- ◆ Ideal for the purification of F(ab')<sub>2</sub>, Fab and scFV fragments containing kappa light chains
- ◆ Great to purify kappa monoclonal antibodies directly from media supplemented with FCS
- ◆ Purification of IgG, IgM, IgA, IgE, IgD containing kappa light chains from serum, ascites and hybridoma supernatant
- ◆ Antibodies sensitive to alkaline pH

Product	Cat.#	Qty
Affarose rProtein L	UP52746A	2 ml
	UP52746C	0.5 ml

Uptima recombinant protein L is from *E. coli* and is also available biotin or HRP conjugated.

Product	Unlabeled	Qty
rProtein L	UP56874A	1 mg
	UP56874B	5 mg
	<b>Biotin</b>	
	UPBI0300	1 ml
	<b>HRP</b>	
	UPL79611	1 ml

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### UptiSpin Protein A and UptiSpin Protein G

*The simplest way to purify antibodies*

The simplest way to purify antibodies from serum, ascites and tissue culture supernatant. In minutes the UptiSpin allow the recovery of high levels of pure antibodies.

- ◆ Convenient and easy to use
- ◆ Fast
- ◆ Cost effective : less than 1 Euro to purify 1 mg
- ◆ Efficient : Proteins are sufficiently pure for further applications.

UptiSpin Protein A & G kits are designed for simple, rapid and multiple antibody purification from serum, ascites and tissue culture supernatant derived from static cultures and bioreactors.

Uptima purification kits incorporate convenient, ready to use, single step affinity spin columns, offering the researcher total protein purification solutions.

#### Convenient and easy-to-use

- Simply insert the pre-packed chromatography resin plug into the device and place it in the centrifuge.
- No mess, no filling columns, no attachment of accessories, no pumps and no lengthy equilibration.
- Kits are supplied with a comprehensive technical and application-supported handbook, a step-wise laminated protocol card and all necessary buffers for rapid and simple antibody purification.

#### Rapid

- The purification protocols provided in the operating handbook permit fast recovery of pure antibodies.
- Multiple samples can be processed at the same time, allowing rapid screening for antibody production.

#### Key Features

Centrifugal spin column format

Uses centrifugal g force as driving force

Many samples can be centrifuged in parallel

Economical

Works from mg to 100's of mg range

#### Cost-effective

- UptiSpin columns can be used by any laboratory with access to a centrifuge.
- The columns are re-usable. Each Mini spin column can be used up to 3 times and each Midi spin column can be used up to 5 times.
- As research budgets are always under pressure, Uptima kits offer a cost-effective alternative.

#### High Performance

- Uptima chromatography spin columns will typically bind the target antibody with greater than 90% recovery.
- Choice of both Protein A & G for complete coverage of antibody subclasses and host species.
- Binding capacity : mini 1 mg/plug  
midi 20 mg/plug

#### Benefits

**Simple to use.**  
**No HPLC or FPLC equipment has to be used. Every lab has a centrifuge.**

**Extremely fast. Separation times are short. No hold up volume or spluttering. High recovery during fast elution step.**

**Multiple samples can be purified at the same time.**

**Low cost purification. Less than 1 Euro per mg IgG\* (\*5 times re-use of Midi).**

**Variable volume and amounts can be loaded and recovered with ease.**  
**Typical amount of rabbit IgG purified per Midi kit = 400 mg (4 plugs)**  
**(> 20 mg rabbit IgG/plug in one run ;**  
**> 10 mg rat IgG/plug/run).**



Loading the plug into the spin column



It is now ready for pre-equilibration with binding buffer followed by centrifugation

# Separation techniques (Proteins)

## Affinity Chromatography

Product	Cat.#
<b>PROTEIN A UptiSpin</b>	
<b>MINI</b>	
Protein A Mini Sample Kit : 2 A Plugs (includes buffers)	UPBB9720
Protein A Mini Kit – 16 A Plugs (includes buffers)	UPBB9730
Protein A Mini Bulk Pack – 48 A plugs (does not include buffers)	UPBB9740
<b>MIDI</b>	
Protein A Midi Kit – 4 Midi A Plugs (includes buffers)	UPBB9750
Protein A Midi Bulk Pack – 12 A Plugs (does not include buffers)	UPBB9760
<b>PROTEIN G UptiSpin</b>	
<b>MINI</b>	
Protein G Mini Sample Kit : 2 G Plug (includes buffers)	UPBB9770
Protein G Mini Kit – 16 G Plugs (includes buffers)	UPBA7770
Protein G Mini Bulk Pack – 48 G plugs (does not include buffers)	UPBB9780
<b>MIDI</b>	
Protein G Midi Kit – 4 Midi G Plugs (includes buffers)	UPBB9790
Protein G Midi Bulk Pack – 12 G Plugs (does not include buffers)	UPBB9800
<b>STARTER PACK A and G (Mini Only)</b>	
Starter Pack: 2 A and 2 G Mini Plugs (does not include buffers)	UPBB9820

### UptiSpin Protein A and G Vacuum kits

#### High Speed purifications

The UptiSpin Protein A & G Vacuum kits are designed to concentrate and purify antibodies from large volumes of dilute cell culture supernatants (up to 2 L) using vacuum as the driving force.

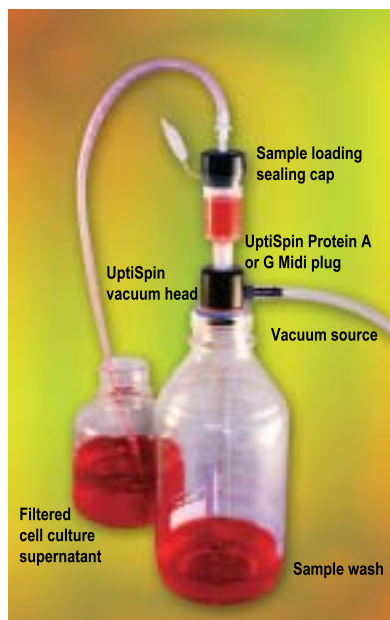
#### Features :

- ◆ Uses universal 50 ml collection tubes and standard glass laboratory bottles up to 2 l
- ◆ Works under low or high vacuum (capture efficiency is enhanced at **lower vacuum**)
- ◆ Simple to set up and easy to use
- ◆ Ultra-filtration concentrators to desalt and concentrate the purified monoclonal antibody
- ◆ Can be performed at room temperature or in cold room

The major benefits is as the driving force is vacuum it permits large volumes of dilute samples to be continuously loaded on to the columns and the centrifuge is not needed.

#### Applications :

- ◆ High speed, hands-free concentration and purification of large volumes of dilute cell culture supernatant without the need of a centrifuge.



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Description	Cat.#	No. of purifications
UptiSpin Protein A Midi Vacuum Kit with Vacuum Head (4 columns, UF concentrators, Buffers)	UPBM8140	20
UptiSpin Protein G Midi Vacuum Kit with Vacuum Head (4 columns, UF concentrators, Buffers)	UPBM8150	20
UptiSpin Protein A Midi Vacuum Kit without Vacuum Head (4 columns, UF concentrators, Buffers)	UPBM8220	20
UptiSpin Protein G Midi Vacuum Kit without Vacuum Head (4 columns, UF concentrators, Buffers)	UPBM8240	20
UptiSpin Protein A Bulk Pack (12 columns only)	UPBM8260	60
UptiSpin Protein G Bulk Pack (12 columns only)	UPBM8270	60
UptiSpin Vacuum Accessory pack containing two complete sets of O-rings (6 x O-rings) plus 6 mm and 8 mm hose barb connectors	UPBM8280	1 unit
Protein A Buffer Pack (Buffers only)	UPBM8360	1 unit
Protein G Buffer Pack (Buffers only)	UPBM8370	1 unit
UptiSpin Vacuum Head and a sample loading sealing cap	UPBM8190	1 unit

### Immobilization of antibodies and peptides for affinity purifications

*Easy immobilization of proteins, through amines !*

#### Ami. R. Gel & Ami. Fast Flow Gel

- ◆ High binding capacity (up to 60 mg/ml BSA)
- ◆ Higher activity of immobilized antibodies than with "oriented" coupling
- ◆ Amine specificity (couple on NH<sub>2</sub>-bearing molecules !)
- ◆ High coupling efficiency (85-99%), whatever is MW and pI !
- ◆ Coupling compatible at pH 3-10, with temperatures 0-40°C
- ◆ Fast coupling (in 20 min. to 2 H)
- ◆ No end-capping necessary
- ◆ Undetectable leaching of ligand (<0.1 ppm)
- ◆ High flow rate (up to 250 cm/h) (200cm/hr for Fast Flow gel)
- ◆ Sanitizable with NaOH, autoclavable

#### Unsurpassed benefits for coupling biomolecules for biotechnological applications !

Preserves protein activity compared to other conventional coupling chemistries (Glutaraldehyde, cyanogen bromide, epoxy, succinimide...)

Ami.R. Gel is favoured in research applications for its efficiency and ease of use. Diagnostic applications also benefit from its high binding efficiency, capacity, and ease of use (especially in manufacturing). In pharmaceutical processing, Ami.R. Gel and Ami.R. Gel Fast Flow replace also conventional activated gels thanks to their extreme stability and cleanability.

Product	Cat.#	Qty
Ami. R. Gel	UP564089	2 ml
	UP56408A	5 ml
	UP56408B	10 ml
Ami. R. Fast Flow Gel	UPR2289A	5 ml
	UPR2289B	10 ml

#### Literature

Grandics, P. et al. 1990 Ann. N.Y. Acad. Sci. 589:148-156  
Thalley, B. and Carroll, S. 1990. Bio-Technology 8:934-938  
Schoepfer, R. et al. 1990. Neuron 5:35-48

# Separation techniques (Proteins)

## Affinity Chromatography

### Protein Immunoprecipitation

#### ImmunoCatcher™

*The complete system for Protein Immuno-Precipitation*

ImmunoCatcher is a complete system that comes with all the components needed for solubilization and immunoprecipitation of proteins. The system utilizes a unique, low protein-binding spin filter for rapid separation, washing and elution of antibody-antigen complexes bound to resin containing immobilized Protein A and Protein G. This technology eliminates tedious repeated washing and re-suspension, as well as the inconvenience associated with loose resin. Multiple preparations of protein antigens can be immunoprecipitated and washed simultaneously, and the samples are ready for analysis in less than 30 minutes. Using the ImmunoCatcher system, you can perform more assays and obtain cleaner proteins in shorter time.

**Fast** : perform 24 or more different assays in less than 30 minutes

**Easy** : eliminate tedious re-suspension and washing of the immunoprecipitates

**Ready-to-use** : include Protein A and Protein G resin, pre-immune serum, lysis solutions, and protease inhibitors

**Lower Background** : remove non-specific binding and contamination effectively using spin filters

**Higher yield** : recover up to 90% of the bound proteins

Applications :

- ◆ Identification of antigens in cell lysate
- ◆ Characterization of post-translational modifications
- ◆ Determination of proteins, nucleic acids or other ligands interacting with the antigens
- ◆ Measurement of protein synthesis and degradation
- ◆ Quantitation of antigen with radioimmunoassay

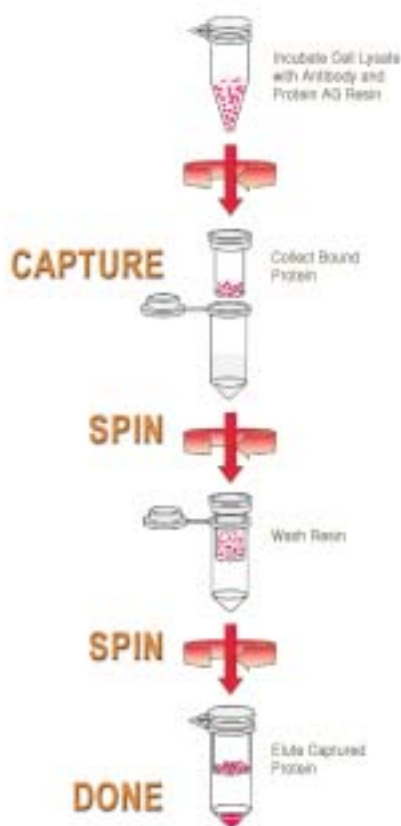
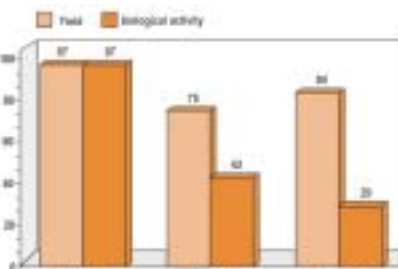


Diagram showing the ImmunoCatcher procedure. Cells are lysed with either Strong or Mild Lysis solutions in the presence of protease inhibitors. The lysate is then incubated with antibody and resin containing immobilized Protein A and Protein G. Following three brief centrifugation steps to collect and wash the resin containing bound antibody-Antigen complexes, the antigens are eluted with SDS-PAGE solution for analysis.

Product	Cat.#	Qty
IMMUNOCatcher™	C02-050	50 assays
Contained		
• 50 centrifuge filters		• 60 ml of strong lysis solution
• 100 collectors		• 0.6 ml of preimmune serum
• 0.6 ml Immobilized protein A/G		• 2 ml of SDS-PAGE sample solution
• 60 ml of mild lysis solution		• Protease inhibitor
IMMUNOCatcher™	C02-020	20 assays
Contained		
• 20 centrifuge filters		• 25 ml of strong lysis solution
• 40 collectors		• 0.25 ml of preimmune serum
• 0.25 ml Immobilized protein A/G		• 1 ml of SDS-PAGE sample solution
• 25 ml of mild lysis solution		• Protease inhibitor
Centrifuge filters and collector	C00-100	
• 100 centrifuge filters		
• 200 collectors		

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Recovery of bound IgG was carried out by using either Uptima mild Elution Medium, 1M acetic acid or chaotropic (3 M NH<sub>4</sub>SCN, pH 7.0) elution buffers. Then yield and activity was determined.



#### Mild IgG elution buffer

Elution under neutral and non-denaturing conditions !

- ◆ Preserves biological activity (>97%)
- ◆ Increased yield (>97%)
- ◆ Increases the longevity of immunoadsorbents
- ◆ 50-100 fold economy per purification cycle cost

Acidic elution not only leads to significant losses of antibody activity, but also causes subtle changes in the IgG conformation. Chaotropic agents exhibit higher bioactivity but with lower yields. Uptima Mild Elution Medium nearly delivers quantitative recovery of antibodies with no detectable loss of bioactivity.

Product	Cat.#	Qty
Mild IgG elution buffer neutral pH	UP38591A	1 L

#### Unwanted proteins removal

Tools to remove unwanted multiple high-abundant proteins with very high specificity  
To know more about this new range of products, please refer to **Sample preparation (page B180)**.

### Magnetic Beads

#### Polystyrene microspheres UptiBeads

Polystyrene microspheres UptiBeads are the most judicious choice of magnetic beads for your usual applications. The excellent rate capture of our super para-magnetic beads are obtained thanks to a very advantageous diameter/ligand load ratio, with a very low dispersal rate size beads. UptiBeads are stable between pH2 and 12, compatible with sonication. They are supplied at 1% suspension in PBS, ready-to use.

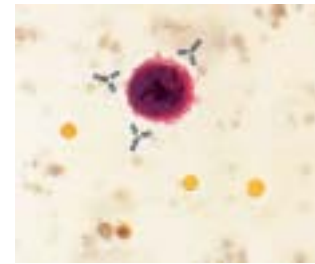
Catalog-available beads diameters are 0.3 and 0.86  $\mu\text{m}$ , and grafted ligands are high affinity anti IgG (all isotypes), anti IgM, Streptavidin and anti Biotin. Ask us for other bead sizes, conditioning, or other ligand of your interest !

- ◆ High binding capacity
- ◆ Monodisperse
- ◆ Excellent colloidal stability
- ◆ Stable
- ◆ The most advantageous specific surface/magnetic capacity

Diameter ( $\mu\text{m}$ )	% ferrite	Density (g/cm <sup>3</sup> )	Particular surface area (cm <sup>2</sup> )	Number of beads (nb/ml)
0.3 $\mu\text{m}$	40%	1.54	$3.0 \times 10^{-9}$	$4.2 \times 10^{10}$
0.86 $\mu\text{m}$	55%	1.54	$3.1 \times 10^{-8}$	$1.6 \times 10^{10}$

Applications :

- ◆ **Cell Biology**
  - Depletion of cells (negative selection)
  - Isolation of cells and bacteria (positive selection)
  - Concentration of cells and bacteria
  - Lateral flow tests (visualization of cell moves)
- ◆ **Diagnostic**
  - Immuno-assays (enzymatic, colorimetric, or chemiluminescent detections) (in tube, in microplates)
  - Agglutination tests (on slides...)
  - Immunoanalysis
- ◆ **Molecular Biology**
  - Purification of Igs (i.e. immune IgGs)
  - Immuno-precipitation
  - Immuno-capture
  - Purification (proteins, complexes, receptors...)
  - Isolation of mRNA et DNA
  - Hybridization of nucleic acids



Specificity	Diameter ( $\mu\text{m}$ )	Binding capacity (mg IgG/g particules)	Cat.# 2 ml	Cat.# 10 ml	Diameter ( $\mu\text{m}$ )	Binding capacity (mg IgG/g particules)	Cat.# 2 ml	Cat.# 10 ml
Anti- Human IgG	0.3 $\mu\text{m}$	0.3-0.6mg	UPR08640	UPR08641	0.86 $\mu\text{m}$	0.1-0.3 mg	UPR08610	UPR08611
Anti-Mouse IgG	0.3 $\mu\text{m}$	0.4-0.6 mg	UPR08410	UPR08411	0.86 $\mu\text{m}$	0.1-0.3 mg	UPQ99910	UPQ99911
Anti-Rabbit IgG	0.3 $\mu\text{m}$	0.3-0.6 mg	UPR08470	UPR08471	0.86 $\mu\text{m}$	0.5-1 mg	UPR08460	UPR08461
Anti-Rat IgG	0.3 $\mu\text{m}$	0.3-0.6 mg	UPR08600	UPR08601	0.86 $\mu\text{m}$	0.2-0.4 mg	UPR08590	UPR08591
Anti-Human IgM	0.3 $\mu\text{m}$	0.75-1 mg	UPR08990	UPR08991	0.86 $\mu\text{m}$	0.1-0.3 mg	UPR08980	UPR08981
Anti-Mouse IgM	0.3 $\mu\text{m}$	2-3 mg	UPR08440	UPR08441	0.86 $\mu\text{m}$	0.75-1.25 mg	UPR08420	UPR08421
Anti-Rabbit IgM	0.3 $\mu\text{m}$	nd	UPR08580	UPR08581	0.86 $\mu\text{m}$	nd	UPR08480	UPR08481
Streptavidine	0.3 $\mu\text{m}$	3-4 mg	UPR09030	UPR09031	0.86 $\mu\text{m}$	2-3 mg	UPR09020	UPR09021
Anti Biotin	0.3 $\mu\text{m}$	3-4 mg	UPR09010	UPR09011	0.86 $\mu\text{m}$	2-3 mg	UPR09000	UPR09001

# Separation techniques (Proteins)

## Affinity Chromatography

### Technical tip

Cobalt shows the higher selectivity towards poly-His sequence but the lowest loading capacity : it is the ion of choice when your recombinant protein is of high value and under present in your sample.

At the opposite, Copper shows the highest loading capacity but the lowest selectivity.

Nickel and Zinc are more often use since they show intermediate selectivity and loading capacity.

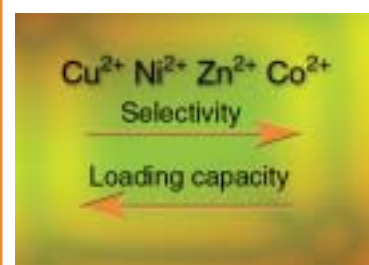
#### High density :

higher is the density of the ions more recombinant proteins will be purify. However in the same time more unwanted proteins are bound.

#### Low density :

qualitatively the purification is quite perfect.

For more convenience we offer chelate test kits as screening tools. Thanks to them you are able to find the right ion and density for your application.



## Recombinant proteins purification - IMAC

IMAC (Immobilized Metal Affinity Chromatography) is based on the interaction between certain superficial protein residues (histidines, cysteines and to a lesser extent tryptophans), and transition metal cations forming chelates ( $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ ).

#### Affarose format :

Ligands are covalently bound to the matrice, a modified agarose called Affarose, to ensure minimal leaching of ligand, excellent stability and to allow re-use.

#### UptiSpin format (ready-to-use kit) :

Designed for simple, rapid and multiple recombinant proteins purification.

#### Affarose IMAC

##### His-Tag Recombinant proteins purification on columns

Our Affarose IMAC are activated with different metal ions at different density. Ions show more or less affinity with the poly-His residues and their loading capacity are also different. Playing on these 2 features offers the possibility to get the right affinity resin for a specific application.

#### Features :

- ◆ Four different ions
- ◆ Two different ions densities

#### Chelate Agarose beads

Bead (geometry, size)	Spherical, Standard : 40-180 $\mu\text{m}$ (approx.)
Cross-linked	Yes
Autoclavable	121°, 30'
Agarose %	6%
Activating Agent	Epichlorohydrin
Ligand	Imminodiacetic Acid (IDA)
IDA Matrix	Stable in strong acid and basic solutions
Storage Temp.	2-8°C
Antimicrobial Agent	20 % Ethanol

Product Name	High Density IDA-affarose			Low Density IDA-affarose		
	Ni-Charged	Zn-Charged	Co-Charged	Cu-Charged	Ni-Charged	Zn-Charged
Binding/LoadingCapacity ( $\mu\text{mol Me}^{2+}/\text{ml gel}$ )		20 – 40			5 - 20	

# Separation techniques (Proteins)

## Affinity Chromatography

Product	Cat.#	Qty
<b>Chelate Affarose Beads</b>		
<b>HIGH</b> Density IDA-Affarose	<b>BG7020</b>	5 ml
<b>NICKEL CHARGED</b>	<b>BG7021</b>	10 ml
	<b>BG7022</b>	25 ml
<b>LOW</b> Density IDA-Affarose	<b>BG7030</b>	5 ml
<b>NICKEL CHARGED</b>	<b>BG7031</b>	10 ml
	<b>BG7032</b>	25 ml
<b>HIGH</b> Density IDA-Affarose	<b>BG7040</b>	5 ml
<b>ZINC CHARGED</b>	<b>BG7041</b>	10 ml
	<b>BG7042</b>	25 ml
<b>LOW</b> Density IDA-Affarose	<b>BG7050</b>	5 ml
<b>ZINC CHARGED</b>	<b>BG7051</b>	10 ml
	<b>BG7052</b>	25 ml
<b>HIGH</b> Density IDA-Affarose	<b>BG7060</b>	5 ml
<b>COBALT CHARGED</b>	<b>BG7061</b>	10 ml
	<b>BG7062</b>	25 ml
<b>LOW</b> Density IDA-Affarose	<b>BG7070</b>	5 ml
<b>COPPER CHARGED</b>	<b>BG7071</b>	10 ml
	<b>BG7072</b>	25 ml

### Chelate Test Kits

**Nickel Chelate kit** **BG7080**

Includes 2ml **HIGH** Density IDA-Affarose **Ni** Charged

2ml **LOW** Density IDA-Affarose **Ni** Charged

**Zinc Chelate kit** **BG7090**

Includes 2ml **HIGH** Density IDA-Affarose **Zn** Charged

2ml **LOW** Density IDA-Affarose **Zn** Charged

**High Density Chelat kit** **BG7100**

Includes 2ml **HIGH** Density IDA-Affarose **Metal Free**

2ml **HIGH** Density IDA-Affarose **Ni** Charged

2ml **HIGH** Density IDA-Affarose **Zn** Charged

2ml **HIGH** Density IDA-Affarose **Co** Charged

**Low Density Chelate kit** **BG7110**

Includes 2ml **LOW** Density IDA-Affarose **Metal Free**

2ml **LOW** Density IDA-Affarose **Ni** Charged

2ml **LOW** Density IDA-Affarose **Zn** Charged

2ml **LOW** Density IDA-Affarose **Cu** Charged



# Separation techniques (Proteins)

## Affinity Chromatography



### UptiSpin IMAC

*Ready-to-use kits for the purification of His-Tag recombinant proteins*

In the next few years, more than 100 000 proteins will be characterised. This huge demand creates a need for new tools to rapidly screen, prepare, purify, isolate, concentrate and desalt protein samples prior to further analysis. UptiSpin IMAC has been developed to answer this.

#### Benefits of the UptiSpin IMAC

- ◆ **Speed :**  
Isolated, purified, concentrated sample in less than 45 minutes for the Midi and less than 20 min for the Mini. Multiple purifications can be performed simultaneously for rapid screening of recombinant protein expression.
- ◆ **Performance :**  
Bind 0.1 mg-20 mg of 6xHis-tagged protein, elute with >90% recovery with high purity and yield. Devices are re-usable.
- ◆ **Convenience :**  
Just spin - only a centrifuge is required.
- ◆ **Price :**  
Spin columns substantially cheaper than research grade FPLC column. Purification at less than 1 Euro/mg recombinant protein !

UptiSpin IMAC are ideal for low-medium throughput expression scouting, automated and manual semi-preparative and preparative purification.

UptiSpin Metal Chelate Mini and Midi kits are designed for simple, complete and rapid 6xHis-tagged recombinant protein purification from bacterial cells, insect vectors, mammalian cells and yeast under native or denaturing conditions.

The kits incorporate patented, single step and affinity spin columns, clear and unambiguous procedures, desalting/buffer exchange columns and ready-to-use buffers. Finally, UptiSpin Metal Chelate kits are ideal for screening expression clones for high levels of 6xHis-tagged proteins, scouting mutant clones for recombinant protein expression and multiple small-scale and preparative purification of 6xHis-tagged proteins for activity and structural studies.

Convenient & easy-to-use :

- ◆ Simply insert the pre-packed chromatography resin plug into the device and place it in the centrifuge.
- ◆ No mess, no filling columns, no attachment of accessories, no pumps and no lengthy equilibration.
- ◆ Kits are supplied with a comprehensive technical and application-supported handbook, a step-wise laminated protocol card and all necessary buffers for rapid and simple recombinant protein purification.

Rapid :

- ◆ The purification protocols provided in the operating handbook permit fast recovery of pure recombinant protein.
- ◆ Multiple samples can be processed at the same time, allowing rapid screening for 6xHis-tagged proteins.
- ◆ No hold-up volume or spluttering. High recovery during the fast elution step.

Cost-effective :

- ◆ UptiSpin columns can be used by any laboratory with access to a centrifuge.
- ◆ The UptiSpin columns are re-usable. Each Mini spin column can be used up to a minimum of 2 times and each Midi spin column can be used up to a minimum of 2 times without nickel re-charging.
- ◆ As research budgets are always under pressure, UptiSpin kits offer a cost-effective alternative.

### Performance :

- ◆ UptiSpin chromatography spin columns will typically bind the target recombinant protein with greater than 90% recovery.
- ◆ UptiSpin spin columns can purify and concentrate the protein of interest in a single step and with high reproducibility (low % CV).
- ◆ Proteins are sufficiently pure for structural and activity-based studies or for raising antibodies.

Kit	MINI	MIDI
Max. sample volume per load	0.65 ml, fixed angle rotor	20 ml, swing bucket rotor
Charged metal ion	Ni <sup>2+</sup>	Ni <sup>2+</sup>
Typical binding capacity per use	1 mg 6xHis-tagged protein	10 mg 6xHis-tagged protein
Min. number of uses per plug	2 (assuming no further Ni <sup>2+</sup> charging)	2 (assuming no further Ni <sup>2+</sup> charging)
Supporting Proteus matrix	Covalently coupled to agarose resin	Covalently coupled to agarose resin
Resin bed volume	0.23 ml	1.6 ml
Bead size range	45-165 µm	45-165 µm
Storage temp. for resin plugs	2-8 °C	2-8 °C
Recommended working pH	pH 2.0-12.0	pH 2.0-12.0
Chemical stability	High	High
Plastic construction	Polypropylene	Polypropylene
Time to purity	15 min	48 min
Color coded end-caps	Black	Black

### UptiSpin IMAC Mini spin column kit contents :

- 24 x UptiSpin column plugs containing Ni-IDA agarose resin.
- 24 x UptiSpin columns (0.65 ml capacity in a fixed angle rotor).
- 48 x 2.2 ml centrifuge tubes.
- 24 x 10 kDa MWCO ultrafiltration spinners.
- 1 x 250 ml 5 x PBS buffer (buffer A) bottle.
- 1 x 150 ml 1 x imidazole buffer (buffer B) bottle.
- Plug insertion tool.
- Comprehensive handbook.
- Ultrafiltration spinner instruction sheet.
- Laminated protocol card.

### UptiSpin IMAC Midi spin column kit contents :

- 8 x UptiSpin spin column plugs containing Ni-IDA agarose resin.
- 8 x UptiSpin spin columns (20 ml capacity in a swing bucket rotor).
- 12 x 50 ml centrifuge tubes.
- 8 x 10 kDa MWCO ultrafiltration spinners.
- 1 x 250 ml 5 x PBS buffer (buffer A) bottle.
- 1 x 150 ml 1 x imidazole buffer (buffer B) bottle.
- Plug insertion tool.
- Comprehensive handbook.
- Ultrafiltration spinner instruction sheet.
- Laminated protocol card.

### Metal Chelate UptiSpin

#### MINI

Metal Chelate Mini Sample Kit - 8 Mini MC Plugs (includes buffers)	UPBB9580
Metal Chelate Mini Kit - 24 MC Plugs (includes buffers)	UPBB9600
Metal Chelate Mini Pack - 24 MC plugs (includes buffers, <b>does not include UF concentrators</b> )	UPBB9620
Metal Chelate Mini Bulk Pack ( <b>does not include buffers or UF concentrators</b> )	UPBB9630

#### MIDI

Metal Chelate Midi Kit - 8 MC plugs (includes buffers)	UPBB9640
Metal Chelate Midi Pack - 8 MC plugs (includes buffers, <b>does not include UF concentrators</b> )	UPBB9650
Metal Chelate Midi Bulk Pack - 24 MC Plugs ( <b>does not include buffers or UF concentrators</b> )	UPBB9660

# Separation techniques (Proteins)

## Affinity Chromatography



### Affinity chromatograph test kits :

#### Toyoscreen TOYOPEARL®

Your fast scouting tool

Toyoscreen - Fast Screening and speedy Set-up of Separation Conditions !  
Find the right resin for your intended application by testing Toyopearl® process resins !

Toyopearl is a methacrylic polymer incorporating high mechanical stability. Resins are available as non-functionalized «HW-» series resins for SEC or derivatised with surface chemistries for alternative modes of chromatography such as IEC (Ion Exchange Chromatography), HIC (Hydrophobic Interaction Chromatography) and AFC (Affinity Chromatography).

- ◆ Short scouting runs
- ◆ Easy to use
- ◆ Gives you the selectivity you need
- ◆ Find the appropriate capacity

It is not an easy job to find the right resin, even when screening materials, just from one resin supplier.

As physical and chemical properties of proteins are so complex, the separation efficiency of a resin is not only a simple function of the ligand. Other determining parameters are : particle size distribution, pore size distribution, pore structure, ligand density and surface hydrophobicity. So it is wise for a resin supplier to provide different grades of resins to the customer.

Toyopearl is produced with four different particle sizes and three different pore sizes. So selection of a particular resin requires testing of, in this case, 12 columns for one ligand. This resin evaluation process is a time consuming optimisation process in a multidimensional parameter space.

The screening of different resins can be easily performed by using **pre-packed small columns** with the same chromatographic performance.

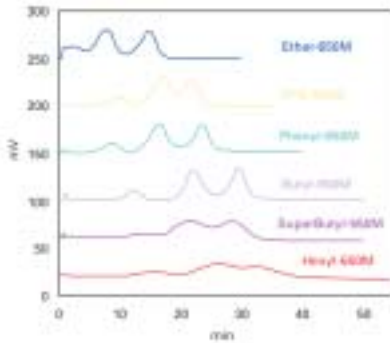


Figure 1  
Ligand Screening of Toyopearl HIC Resins on a Standard Protein

A ligand screening for Toyopearl HIC resins for the separation of standard proteins is shown in Figure 1.

A ToyoScreen kit consists of small 1 or 5 ml columns and holder). The columns can be reused several times and can be connected to all HPLC and Bio-HPLC systems.

Screening of operational Parameters :

In process development a fast change in elution- , equilibration- and purification conditions is required.

Parameters like pH, salt content, gradient volume, flow rate and loading has to be changed many times to find optimal conditions. Common factorial designs are used to minimise the experimental efforts. A prerequisite is the reproducibility of the experiments which is strongly dependent from a good quality of the testing columns which can not be always assured by self packed columns. For this, ToyoScreen is an ideal tool.

The effect of gradient volume and flow rate for the resolution for a ToyoScreen SuperQ-650M column is shown in Figure 2.

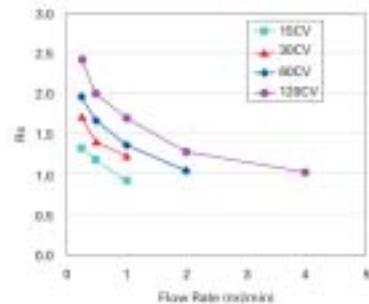


Figure 2  
Change in Resolution in Correlation of Gradient Volume and flow rate.

Summary :

- ◆ Fast screening tool to find the best Toyopearl resin for your application
- ◆ Easy connection to all HPLC and FPLC systems
- ◆ Small columns for short cycle times simulating your production in downscale with the real process particles prepacked in 1ml and 5 ml ready to use columns

#### Toyoscreen HIC Series (Hydrophobic Interaction Chromatography)

Ligand	Hydrophobicity	Particle Size
Ether-650M	Low	40-90 µm
PPG-600M		40-90 µm
Phenyl-650M		40-90 µm
Butyl-650M		40-90 µm
SuperButyl-550C		50-150 µm
Hexyl-650C	High	50-150 µm

B.178

### ToyoScreen IEC Series (Ion Exchange Chromatography)

Ligand	Ion exchange capacity	Particle Size
DEAE-650M	0.08-0.12 eq/l-gel	40-90 µm
SuperQ-650M	0.20-0.30 eq/l-gel	40-90 µm
QAE-550C	0.28-0.38 eq/l-gel	50-150 µm
CM-650M	0.08-0.12 eq/l-gel	40-90 µm
SP-650M	0.13-0.17 eq/l-gel	40-90 µm
SP-550C	0.14-0.18 eq/l-gel	50-150 µm



### ToyoScreen AFC Series (Affinity Chromatography)

Ligand Particle	Size
AF-Chelate-650M	40-90 µm
AF-Blue HC-650M	40-90 µm
AF-Red-650M	40-90 µm
AF-Heparin HC-650M	40-90 µm



Description	Cat.#	Contents
ToyoScreen Holder	21400	1 u

#### Serie IEC

ToyoScreen IEC	21396	1 ml	6 Grades * 1 Column
ToyoScreen IEC	21397	5 ml	6 Grades * 1 Column
ToyoScreen C-IEC	21394	1 ml	3 Grades * 2 Columns
ToyoScreen C-IEC	21395	5 ml	3 Grades * 2 Columns
ToyoScreen A-IEC	21392	1 ml	3 Grades * 2 Columns
ToyoScreen A-IEC	21393	5 ml	3 Grades * 2 Columns
ToyoScreen SP-550C	21370	1 ml	6 Columns
ToyoScreen SP-550C	21371	5 ml	6 Columns
ToyoScreen SP-650M	21368	1 ml	6 Columns
ToyoScreen SP-650M	21369	5 ml	6 Columns
ToyoScreen CM-650M	21366	1 ml	6 Columns
ToyoScreen CM-650M	21367	5 ml	6 Columns
ToyoScreen QAE-550C	21364	1 ml	6 Columns
ToyoScreen QAE-550C	21365	5 ml	6 Columns
ToyoScreen SuperQ-650M	21362	1 ml	6 Columns
ToyoScreen SuperQ-650M	21363	5 ml	6 Columns
ToyoScreen DEAE-650M	21360	1 ml	6 Columns
ToyoScreen DEAE-650M	21361	5 ml	6 Columns

#### Serie AFC

ToyoScreen AF-Heparin HC-650M	21390	1 ml	6 Columns
ToyoScreen AF-Heparin HC-650M	21391	5 ml	6 Columns
ToyoScreen AF-Red-650M	21388	1 ml	6 Columns
ToyoScreen AF-Red-650M	21389	5 ml	6 Columns
ToyoScreen AF-Blue HC-650M	21386	1 ml	6 Columns
ToyoScreen AF-Blue HC-650M	21387	5 ml	6 Columns
ToyoScreen AF-Chelate-650M	21384	1 ml	6 Columns
ToyoScreen AF-Chelate-650M	21385	5 ml	6 Columns

#### Serie HIC

ToyoScreen HIC	21398	1 ml	6 Grades * 1 Column
ToyoScreen HIC	21399	5 ml	6 Grades * 1 Column
ToyoScreen SuperButyl-550C	21382	1 ml	6 Columns
ToyoScreen SuperButyl-550C	21383	5 ml	6 Columns
ToyoScreen PPG-600M	21380	1 ml	6 Columns
ToyoScreen PPG-600M	21381	5 ml	6 Columns
ToyoScreen Hexyl-650C	21378	1 ml	6 Columns
ToyoScreen Hexyl-650C	21379	5 ml	6 Columns
ToyoScreen Butyl-650M	21376	1 ml	6 Columns
ToyoScreen Butyl-650M	21377	5 ml	6 Columns
ToyoScreen Phenyl-650M	21374	1 ml	6 Columns
ToyoScreen Phenyl-650M	21375	5 ml	6 Columns
ToyoScreen Ether-650M	21372	1 ml	6 Columns
ToyoScreen Ether-650M	21373	5 ml	6 Columns