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Protein G HP **SpinTrap** / Ab **SpinTrap**

Product Booklet

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1 Introduction

Product codes

28903134

28408347

Important

Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

Storage

Store at 4°C to 8°C.

2 Background

Protein G HP SpinTrap™ columns consist of small scale pre-packed spin columns withProtein G Sepharose™ HP High Performance.

The columns are designed for two different applications:

- Enrichment of target proteins
- Purification of antibodies

This instructions provide background information, protocols, and general useful information for both applications.

Ab SpinTrap contains:

- 50 prepacked Protein G HP SpinTrap columns
- Instructions for use

3 General handling of the SpinTrap column

Lids and bottom caps

Lids and bottom caps are used during the incubation and elution but not during equilibration and washing. Before centrifugation, remove the bottom cap and slightly open the screw cap lid (twist the cap lid ~90º counterclockwise).

Bottom cap removal

Twist the bottom cap off the SpinTrap column, before dispensing liquid into the column. Remember to save the bottom cap.

Incubation

Make sure that the medium is fully suspended before incubating with end-over-end mixing. All incubations should normally be performed at room temperature. However, incubations may be performed at lower temperatures when a slower process is preferable (see *Chapter 4 Antibody purification, on page 6* for further information).

After centrifugation

Immediately after centrifugation, re-insert the bottom cap into the bottom of the SpinTrap column (before the incubation and elution steps).

Liquid collection

After each step, place the SpinTrap column in a fresh 2 mL microcentrifuge tube (not included) for liquid collection.

Elution

For the elution steps, mix by manually inverting the SpinTrap column.

4 Antibody purification

Purpose

The Protein G HP SpinTrap columns are designed for rapid small-scale antibody purification of multiple samples in parallel, for example in antibody screening experiments.

Principle

Protein G Sepharose HP has a high protein binding capacity and is compatible with all commonly used buffers in antibody purification. The column can be used with a standard micro centrifuge and one purification takes less than 20 mins. Cell culture supernatants, as well as serum samples, may be directly applied to the column without prior clarification.

4.1 Advice on handling

Optimization of parameters

The parameters for antibody purification may require optimization. Examples of parameters which may require optimization are:

- sample pre-treatment
- amount of antibody to be purified
- incubation time
- choice of buffers
- number of washes

Sample pre-treatment

Antibodies from several species can be purified with Protein G Sepharose High Performance.

IgG from many species has a medium to strong affinity for protein G at approximately pH 7.0, see *Section 4.2 Antibody binding to protein A and protein G, on page 9*.

The sample should have a pH around 7 before applying to a spin column. It is therefore important to check the pH of the sample, and adjust it as necessary before applying the sample to the column.

Choice of buffers

The following buffers are recommended.

Binding buffer:	20 mM sodium phosphate, pH 7.0
Elution buffer:	0.1 M glycine-HCl, pH 2.7
Neutralizing buffer:	1 M Tris-HCl, pH 9.0

Note: *Use high-purity water and chemicals for buffer preparation.*

- Recommended buffers can be easily prepared using Ab Buffer Kit, see *Chapter 7 Ordering information, on page 22*
- Protein G Sepharose High Performance binds IgG over a wide pH range with a strong affinity at neutral pH. To elute the IgG, it is necessary to lower the pH to about 2.5 to 3.0 depending on the antibody.
- As a safety measure to preserve the activity of acid-labile IgGs, we recommend the addition of 1 M Tris-HCl, pH 9.0, to tubes used for collecting IgG-containing fractions (60 to 200 µL/mL eluted fraction). In this way, the final pH of the sample will be approximately neutral.

Antibody recovery

- If the pH of the sample is too low the antibody may have low binding to Protein G Sepharose High Performance matrix. Ensure that the pH is approximately 7.
- If the sample contains more antibody than the column has capacity for, the recovery will decrease. Decrease the amount of sample added to each column.

4.2 Antibody binding to protein A and protein G

Relative binding strengths for protein A and protein G

Species	Subclass	Protein A binding	Protein G binding
Human	IgA	variable	-
	IgD	-	-
	IgD	-	-
	IgG_1	++++	++++
	IgG_2	++++	++++
	IgG_3	-	++++
	IgG_4	++++	++++
	IgM	variable	-
Avian egg yolk	IgY	-	-
Cow		++	++++
Dog		++	+
Goat		-	++
Guinea pig	IgG_1	++++	++
	IgG_2	++++	++
Hamster		+	++
Horse		++	++++
Koala		-	+
Llama		-	+
Monkey (rhesus)		++++	++++
Mouse	IgG_1	+	++++
	IgG_{2a}	++++	++++
	IgG_{2b}	+++	+++
	IgG_3	++	+++
	IgM	variable	-
Pig		+++	+++
Rabbit		++++	+++
Rat	IgG_1	-	+

Species	Subclass	Protein A binding	Protein G binding
	IgG_{2a}	-	++++
	IgG_{2b}	-	++
	IgG_3	-	++
Sheep		+/-	++

++++	=	strong binding
++	=	medium binding
-	=	weak or no binding

Antibody purification protocol

The protocol may need optimization for your application, see *Section 4.1 Advice on handling, on page 7*.

Prepare 2 collection tubes per sample for eluted fractions, each containing 30 µL neutralizing buffer.

Remove storage solution

Step	Action
1	Resuspend medium.
2	Remove the bottom cap from the column with help of the plastic bottom cap removal tool. Save the bottom cap.
3	Remove the storage solution by centrifugation for 30 s at 70–100 × g.
4	Proceed with the next part of the protocol.

Equilibrate

Step	Action
1	Add 600 µL binding buffer.
2	Centrifuge for 30 s at 70–100 × g.
3	Proceed with the next part of the protocol.

Bind antibody

Step	Action
1	Add maximum 600 µL of the antibody solution.
2	Secure the top cap tightly and incubate for 4 min while gently mixing.
3	Centrifuge for 30 s at 70–100 × g.
	Note: *Several sample applications can be made subsequently as long as the capacity of the column is not exceeded.*
4	Proceed with the next part of the protocol.

Wash

Step	Action
1	Add 600 µL binding buffer.
2	Centrifuge for 30 s at 70–100 × g.
3	Add 600 µL binding buffer.
4	Centrifuge for 30 s at 70–100 × g.
5	Proceed with the next part of the protocol.

Elute antibody

Step	Action
1	Add 400 µL of elution buffer and mix by inversion.
2	Place the column in a 2 mL microcentrifuge tube containing 30 µL neutralizing buffer (see step 1).
3	Centrifuge for 30 s at 70 × g and collect the eluate.
4	Place the column in a new 2 mL microcentrifuge tube containing 30 µL neutralizing buffer (see step 1).
5	Centrifuge for 30 s at 70 × g and collect the second eluate.
	Note: *Most of the bound antibody is eluted after two elution steps.*

5 Protein enrichment

Purpose

Protein G HP SpinTrap columns are designed for small-scale protein enrichment for single use, for example for use upstream of gel electrophoresis, liquid chromatography, and mass spectrometry.

Principle

There are two protocols for protein enrichment using Protein G HP SpinTrap columns:

Cross-link protocol

In the cross-link protocol the protein capturing antibodies are covalently bound to the Protein G Sepharose High Performance matrix by using a cross-linking agent.

The protein of interest is enriched from the sample, purified through washings, and eluted from the column whereas the antibody remains bound to the matrix.

Use the cross-link protocol:

- If the desired protein/antigen has similar molecular weight as the heavy or light chain of the antibody, which causes problem with comigration in SDS-PAGE analysis.
- If the antibody interferes with downstream analysis.

Classic protocol

In the classic protocol protein capturing antibodies are immobilized by binding to protein G in the Protein G Sepharose High Performance matrix. The classic protocol requires that the capturing antibody used binds to protein G.

The protein/antigen of interest is enriched from the sample, purified through washings and eluted from the column together with the antibody.

Advice on handling

Optimization of parameters

The optimal parameters for protein enrichment are dependent on the specific antibody-antigen combination. Optimization may be required for each specific antibody-antigen combination to obtain the best results.

Examples of parameters which may require optimization are:

- Sample pre-treatment
- Amount of protein (antigen) to be enriched
- Incubation time

- Choice of buffers
- Number of washes

Sample pre-treatment

- Excessive cellular debris and lipids may clog the column. Clarify the sample by centrifugation or filtration before applying to the SpinTrap column.
- To prevent target protein degradation, inhibition of protease activity may be required (a Protease Inhibitor Mix is available, see *Chapter 7 Ordering information, on page 22*).

Incubation time

At room temperature, the reaction is usually completed within 30 to 60 min. If the binding is performed at 4°C, it can be left overnight.

Choice of buffers

It is recommended to use the listed buffers for the indicated type of protocol. A Protein A/G Buffer Kit is available as an accessory for increased convenience, see *Chapter 7 Ordering information, on page 22*. If optimization is required try to use the alternative buffers.

Cross-link protocol	
Binding buffer:	TBS (50 mM Tris, 150 mM NaCl, pH 7.5)
Wash buffer:	TBS with 2 M urea, pH 7.5
Elution buffer:	0.1 M glycine with 2 M urea, pH 2.9
Cross-link solutions:	• 200 mM triethanolamine, pH 8.9 • 50 mM DMP (Dimethyl pimelimidate dihydrochloride) in 200 mM triethanolamine, pH 8.9 • 100 mM ethanolamine, pH 8.9
Classic protocol	
Binding buffer:	TBS (50 mM Tris, 150 mM NaCl, pH 7.5)
Wash buffer:	TBS
Elution buffer:	2.5% acetic acid
Alternative buffers	
Wash buffer:	• TBS (mild wash) • TBS with 1% octylglucoside, pH 7.5 • 0.1 M triethanolamine, 0.5 M NaCl, pH 9.0
Elution buffers:	• 0.1 M glycine, pH 2.5 to 3.1 • 0.1 M citric acid, pH 2.5 to 3.1 • 2% SDS • 0.1 M ammonium hydroxide, pH 10 to 11

Protein recovery and specific purity

- Improve the specific purity by adding detergent, different salts, and different concentrations of salts to the wash buffer.
- Avoid acidic elution conditions since this may cause low protein yield.
- Minimize impurities that may co-elute with the target protein by adding a preclearing step before the enrichment procedure. For preclearing, use a SpinTrap column that has not been coupled with an antibody. Add the sample and incubate for 0.5 to 4 h. Collect the sample by centrifugation and proceed with the standard protocol using the coupled medium.
- Try alternative buffers, see *Choice of buffers, on page 13*.

- The specific purity may be improved if the SpinTrap column is mixed by inverting several times between the washes in step 6 of the protocol (see *Classic protocol, on page 18*).

Additional options when using the classic protocol

- Incubate the antibody with the sample to form an antibody-antigen complex before applying the sample to the column. The complex is then applied to the column for binding.

Cross-link protocol

The protocol may need optimization for your application, see *Advice on handling, on page 12*.

Remove storage solution

Step	Action
1	Twist off the bottom cap from the column with help of the plastic bottom cap removal tool. Save the bottom cap.
2	Remove the storage solution by centrifugation for 1 min at 150 × g.
3	Proceed with the next part of the protocol.

Equilibrate

Step	Action
1	Add 400 µL binding buffer and centrifuge for 1 min at 150 × g to equilibrate the medium.
2	Perform this step 3 times total.
3	Proceed with the next part of the protocol.

Bind antibody

Step	Action
1	Immediately after equilibration, add 200 µL of the antibody solution (0.5 to 1.0 mg/mL in binding buffer).

Step	Action
2	Fully suspend the medium by manual inversion and incubate with slow, end-over-end mixing for 30 min.
3	Centrifuge for 1 min at 150 × g to remove unbound antibody.
4	Proceed with the next part of the protocol.

Wash

Step	Action
1	Add 400 µL binding buffer and centrifuge for 1 min at 150 × g.
2	Proceed with the next part of the protocol.

Change buffer

Step	Action
1	Add 400 µL triethanolamine and centrifuge for 1 min at 150 × g.
2	Proceed with the next part of the protocol.

Cross-link

Step	Action
1	Add 400 µL DMP in triethanolamine.
2	Fully suspend the medium by manual inversion and incubate with slow, end-over-end mixing for 60 min.
3	Centrifuge for 1 min at 150 × g.
4	Proceed with the next part of the protocol.

Wash

Step	Action
1	Add 400 µL triethanolamine and mix by manual inversion
2	Centrifuge for 1 min at 150 × g.

Step	Action
3	Proceed with the next part of the protocol.

Block

Step	Action
1	Add 400 µL ethanolamine.
2	Mix by manual inversion and incubate end-over-end for 15 min.
3	Centrifuge for 1 min at 150 × g.
4	Proceed with the next part of the protocol.

Remove unbound antibody

Step	Action
1	Add 400 µL elution buffer and centrifuge for 1 min at 150 × g.
2	Proceed with the next part of the protocol.

Wash

Step	Action
1	Add 400 µL binding buffer and centrifuge for 1 min at 150 × g.
2	Perform this step 2 times total.
3	Proceed with the next part of the protocol.

Bind target protein

Step	Action
1	Add 200 µL of sample in binding buffer.
2	Mix by manual inversion. Incubate with slow, end-over-end mixing for 60 min. Centrifuge for 1 min at 150 × g to wash out unbound sample. Collect flowthrough.
3	Proceed with the next part of the protocol.

Wash

Step	Action
1	Add 400 µL wash buffer and centrifuge for 1 min at 150 × g.
2	Perform this step 5 times total.
3	During optimization/trouble shooting: Collect flowthrough.
4	Proceed with the next part of the protocol.

Elute

Step	Action
1	Add 200 µL of desired elution buffer and mix by inversion. Centrifuge for 1 min at **1000 × g**. Perform this procedure 3 times total.
2	Collect the eluates in individual tubes.

Classic protocol

The protocol may need optimization for your application, see *Advice on handling, on page 12*.

Remove storage solution

Step	Action
1	Twist off the bottom cap from the column. Save the bottom cap.
2	Remove the storage solution by centrifugation for 1 min at 150 × g.
3	Proceed with the next part of the protocol.

Equilibrate

Step	Action
1	Add 400 µL binding buffer and centrifuge for 1 min at 150 × g to equilibrate the medium.
2	Perform this step 3 times total.
3	Proceed with the next part of the protocol.

Bind antibody

Step	Action
1	Immediately after equilibration, add 200 µL of the antibody solution (0.5 to 1.0 mg/mL in binding buffer).
2	Fully suspend the medium by manual inversion and incubate with slow, end-over-end mixing for 30 min.
3	Centrifuge for 1 min at 150 × g to remove excessive antibody.
4	Proceed with the next part of the protocol.

Wash

Step	Action
1	Add 400 µL binding buffer and centrifuge for 1 min at 150 × g.
2	Proceed with the next part of the protocol.

Bind target protein

Step	Action
1	Add 200 µL sample in binding buffer.
2	Mix by manual inversion. Incubate with slow, end-over-end mixing for 60 min.
3	Centrifuge for 1 min at 150 × g to wash out unbound sample.
4	During optimization/trouble shooting: Collect flowthrough.
5	Proceed with the next part of the protocol.

Wash

Step	Action
1	Add 400 µL wash buffer and centrifuge for 1 min at 150 × g.
2	Perform this step 5 times.
3	During optimization/trouble shooting: Collect flowthrough.
4	Proceed with the next part of the protocol.

Elute

Step	Action
1	Add 200 µL of desired elution buffer and mix by inversion.
2	Centrifuge for 1 min at **1000 × g**.
3	Perform this procedure 3 times.
4	Collect the eluates in individual tubes.

6 Characteristics

Matrix	Highly cross-linked agarose, 6%
Medium	Protein G Sepharose High Performance
Ligand	Recombinant protein G lacking albumin-binding region
Ligand coupling method	N-hydroxysuccinimide activation
Ligand density	approx. 2 mg protein G/mL medium
Binding capacity[1]	> 1 mg human IgG/column
Average particle size	34 µm
pH stability[2]	3 to 9 (long term) 2 to 9 (short term)
Working temperature	4°C to 30°C
Storage solution	20% ethanol
Storage temp	4°C to 8°C
Column material	Polypropylene barrel, polyethylene frits
Volume, prepacked medium	100 µL
Column volume	800 µL

[1] The binding capacity has been determined using human polyclonal IgG.
[2] pH below 3 is sometimes required to elute strongly bound Ig species. However, protein ligands may hydrolyze at very low pH.

7 Ordering information

Products

Description	Quantity	Product code
Protein G HP SpinTrap	16 columns	28903134
Ab SpinTrap	50 columns	28408347

Related products

Description	Quantity	Product code
Sample Grinding Kit	50 samples	80648337
Protease Inhibitor Mix	1 mL	80650123
Nuclease Mix	0.5 mL	80650142
NHS HP SpinTrap	5 mL medium, 24 columns	28903128
Streptavidin HP SpinTrap	16 columns	28903130
Streptavidin HP MultiTrap™	4 × 96-well filter plates	28903131
Protein A HP SpinTrap	16 columns	28903132
Protein A HP MultiTrap	4 × 96-well filter plates	28903133
Protein G HP MultiTrap	4 × 96-well filter plates	28903135
Collection Plate	5 × 96 well plates	28403943
Ab Buffer Kit	1	28903059
Protein A/G HP SpinTrap Buffer Kit	1	28913567

Literature

Title	Product code
Data File Ab SpinTrap	28902030
Data File Protein G HP SpinTrap, Protein G HP MultiTrap, Protein A/G HP SpinTrap Buffer Kit	28906790
Antibody Purification Handbook	18103746
Affinity Chromatography Handbook	18102229

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