

Protein A HP SpinTrap™

Product Booklet

Introduction

Product code

28903132

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

Storage temperature 4–8°C.

Expiry

24 months.

Background

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

The columns are designed for two different applications:

- Enrichment of target protein.
- Purification of antibodies.

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

Streptavidin HP Protein A HP SpinTrap contains

- 16 prepacked Protein A HP SpinTrap columns.
- 1 plastic bottom cap removal tool
- Instructions for use.

General handling of the Protein A HP 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 ~90° counter-clockwise).
- **Bottom cap removal:** Twist the bottom cap off the Protein A HP 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 must normally be performed at room temperature. However, incubations may be performed at lower temperatures when a slower process is preferable (see the following section for further information).
- **After centrifugation:** Immediately after centrifugation, re-insert the bottom cap into the bottom of the Protein A HP SpinTrap column (before the incubation and elution steps).
- **Liquid collection:** After each step, place the Protein A HP SpinTrap column in a fresh 2 mL microcentrifuge tube (not included) for liquid collection.
- **Elution:** For the elution steps, mix by manually inverting the Protein A HP SpinTrap column.

Antibody purification

Purpose

The Protein A HP SpinTrap columns are designed for rapid smallscale antibody purification of multiple samples in parallel, for example in antibody screening experiments.

Principle

Protein A 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 minutes. Cell culture supernatants, as well as serum samples, may be directly applied to the column without prior clarification.

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 A Sepharose High Performance.

IgG from many species has a medium to strong affinity for Protein A at approximately pH 7.0, see [Antibody binding to Protein A and protein G, on page 2](#).

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 (Product code 28903059).
- Protein A 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 A Sepharose High Performance matrix. Make sure 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.

Antibody binding to Protein A and protein G

Table 1. 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	+	++++

Species	Subclass	Protein A binding	Protein G binding
	IgG _{2a}	++++	++++
	IgG _{2b}	+++	+++
	IgG ₃	++	+++
	IgM	variable	-
Pig		+++	+++
Rabbit		++++	+++
Rat	IgG ₁	-	+
	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 [Optimization of parameters, on page 3](#).

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

Remove storage solution

Step	Action
1	Re-suspend medium.
2	Remove the bottom cap from the column. 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.

Protein enrichment

Purpose

The Protein A 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 A HP SpinTrap columns:

Cross-link protocol

In the cross-link protocol the protein capturing antibodies are covalently bound to the Protein A 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 A in the Protein A Sepharose High Performance matrix. The classic protocol requires that the capturing antibody used binds to protein A.

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

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 Protein A HP SpinTrap column.
- To prevent target protein degradation, inhibition of protease activity may be required (a Protease Inhibitor Mix is available, see [Ordering information, on page 6](#)).

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 [Ordering information, on page 6](#). 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	<ul style="list-style-type: none">• 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	<ul style="list-style-type: none">• TBS (mild wash)• TBS with 1% octylglucoside, pH 7.5• 0.1 M triethanolamine, 0.5 M NaCl, pH 9.0
Elution buffers	<ul style="list-style-type: none">• 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 Protein A HP 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 [Cross-link protocol, on page 4](#).
- The specific purity may be improved if the Protein A HP SpinTrap column is mixed by inverting several times between the washes in step 6 of the protocol (see [Classic protocol, on page 5](#)).

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, [on page 3](#).

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 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.
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-overend 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. Perform this step 2 times total.
2	Proceed with the next part of the protocol.

Bind target protein

Step	Action
1	Add 200 μ L 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 \times g to wash out unbound sample. Collect flowthrough.
3	Proceed with the next part of the protocol.

Wash

Step	Action
1	Add 400 μ L wash buffer and centrifuge for 1 min at 150 \times g.
2	Perform this step 5 times total. (Collect and save washes in case troubleshooting is needed).
3	Proceed with the next part of the protocol.

Elute

Step	Action
1	Add 200 μ L of desired elution buffer and mix by inversion, and centrifuge for 1 min at 1000 \times g. Perform this procedure 3 times total. Collect the eluates in individual tubes.
2	Perform this step 3 times total.

Classic protocol

The protocol may need optimization for your application, see [, on page 3](#).

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 \times g.
3	Proceed with the next part of the protocol.

Equilibrate

Step	Action
1	Add 400 μ L binding buffer and centrifuge for 1 min at 150 \times 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 μ L 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.

Step	Action
3	Centrifuge for 1 min at 150 \times g to remove excessive antibody.
4	Proceed with the next part of the protocol.

Wash

Step	Action
1	Add 400 μ L binding buffer and centrifuge for 1 min at 150 \times g.
2	Proceed with the next part of the protocol.

Bind target protein

Step	Action
1	Add 200 μ L 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 \times g to wash out unbound sample. Collect flowthrough.
4	Proceed with the next part of the protocol.

Wash

Step	Action
1	Add 400 μ L wash buffer and centrifuge for 1 min at 150 \times g.
2	Perform this step 5 times. (Collect and save washes in case troubleshooting is needed).
3	Proceed with the next part of the protocol.

Elute

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

Characteristics

Matrix	Highly cross-linked agarose, 6%
Medium	Protein A Sepharose High Performance
Ligand	Native protein A
Ligand coupling method	N-hydroxysuccinimide activation
Ligand density	approx. 3 mg protein A/mL medium
Binding capacity ¹	> 1 mg human IgG/column
Average particle size	34 μ m
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.

Ordering information

Table 2. Products

Description	Quantity	Product code
Protein A HP SpinTrap	16 columns	28903132

Table 3. 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 G HP SpinTrap	16 columns	28903134
Ab SpinTrap	50 columns	28408397
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 SpinTrap	50 × 100 µL	28408347
Ab Buffer Kit	1	28903059
Protein A/G SpinTrap Buffer Kit	1	28913567

Table 4. Literature

Title	Product code
Data File Protein A HP SpinTrap	28906789
Antibody Purification Handbook	18103746
Affinity Chromatography Handbook	18102229

cytiva.com

Cytiva and the Drop logo are trademarks of Global Life Sciences IP Holdco LLC or an affiliate.

MultiTrap, Sepharose, and Spintrap are trademarks of Global Life Sciences Solutions USA LLC or an affiliate doing business as Cytiva.

All other third-party trademarks are the property of their respective owners.

© 2020–2022 Cytiva

For local office contact information, visit cytiva.com/contact

28906770 AG V:7 02/2022

