cytiva

Protein G HP **MultiTrap**

Instructions for Use

Protein G HP MultiTrap contains

- 4 prepacked Protein G HP MultiTrap™ 96-well filter plates
- Instructions for use

Introduction

Protein G HP MultiTrap is designed for small-scale sample preparation for single use, for example upstream of gel electrophoresis, liquid chromatography and mass spectrometry. MultiTrap may be used with robotic systems or manually, with centrifugation or vacuum.

The plate wells contain Protein G Sepharose™ High Performance columns. The plates are designed for two different applications:

- **Enrichment of target proteins**
- **Purification of antibodies**

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

General handling of the MultiTrap plate

- **Centrifugation/vacuum:** Centrifuge the MultiTrap plates or use vacuum. If vacuum is used, apply 0.15 bar until the wells are empty, then slowly increase the vacuum to -0.3 bar (do not apply more vacuum than -0.5 bar). Turn off the vacuum after approximately 5 sec.
- **Medium:** Mix briefly before removal of liquid in the equilibration, wash and elution steps to increase the efficiency of the step. Incubating on a plate shaker is recommended.
- **Incubation:** During incubation, cover the plate using a sealing tape or an appropriate 96-well cover.
- **Collection plates:** Collection plates are not included and must be ordered separately (see *Ordering information, on page 5*). Remember to change or empty the collection plate between steps.
- **Sample pretreatment:** Excessive cellular debris and lipids may clog the column. Clarify the sample by centrifugation or filtration before applying to the MultiTrap plate MultiTrap plate well.
- **Sample pretreatment:** To prevent target protein degradation, inhibition of protease activity may be required (a Protease Inhibitor Mix is available, see *Ordering information, on page 5*).

Antibody purification

Purpose

The Protein G HP MultiTrap prepacked 96-well plates 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 MultiTrap can be used with a standard centrifuge and one purification takes less than 20 minutes. Cell culture supernatants, as well as serum samples, may be directly applied to the wells without prior clarification.

Advice on handling

Optimization of parameters

The parameters for antibody purification may require optimization.

Examples of parameters which may require optimization are:

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

Sample pretreatment

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 *Antibody binding to Protein A and protein G, on page 2*.

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

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 *Ordering information, on page 5*.

- 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 collection plate 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 MultiTrap wells does not have enough capacity for the amount of sample the recovery will be less than expected. Decrease the amount of sample added to each well.

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$_1$	-	+
	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 *Advice on handling, on page 1*. Please refer to *General handling of the MultiTrap plate, on page 1* for general handling instructions.

Step	Action
1	**Prepare collection plates** For step 6, prepare 2 collection plates for eluted fractions, each containing 15 µl neutralizing buffer per well.
2	**Remove storage solution** **a.** Suspend the medium by gently shaking the plate upside down. **b.** Remove top and bottom seals and place the MultiTrap plate on a collection plate. **c.** Remove the storage solution by centrifugation for 1 min at 70 to 100 × *g*.
3	**Equilibrate** **a.** Add 300 µl binding buffer and mix briefly. **b.** Centrifuge for 30 s at 70 to 100 × *g*.
4	**Bind antibody** **a.** Add maximum 300 µl of the antibody solution. **b.** Incubate for 4 min while gently mixing. **c.** Centrifuge for 30 s at 70 to 100 × *g*. ***Note:*** *Several sample applications can be made subsequently as long as the capacity of the column is not exceeded.*
5	**Wash** **a.** Add 300 µl binding buffer, mix briefly and centrifuge for 30 s at 70 to 100 × *g*. **b.** Perform this step 2 times total.
6	**Elute antibody** **a.** Replace the collection plate with a collection plate prepared in step 1. **b.** Add 200 µl of elution buffer, mix briefly and centrifuge for 30 s at 70 × *g* and collect the eluate. **c.** Perform this procedure 2 times total. ***Note:*** *Most of the bound antibody is eluted after two elution steps.*

Protein enrichment

Purpose

The Protein G HP MultiTrap prepacked 96-well plates 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 MultiTrap prepacked 96-well plates:

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 wells 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 well 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 MultiTrap wells. Clarify the sample by centrifugation or filtration before applying to the MultiTrap well.
- To prevent target protein degradation, inhibition of protease activity may be required (a Protease Inhibitor Mix is available, see *Ordering information, on page 5*).

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. 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 buffers:	• 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 MultiTrap well 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.

Additional options when using the classic protocol

- Incubate the antibody with the sample to form an antibodyantigen complex before applying the sample to the well. The complex is then applied to the wells for binding.

Cross-link protocol

The protocol may need optimization for your application, see Advice on handling. Please refer to *General handling of the MultiTrap plate, on page 1* for general handling instructions.

Step	Action
1	**Remove storage solution** • Suspend the medium by gently shaking the plate upside down. • Remove top and bottom seals and place on a collection plate. • Remove the storage solution by centrifugation for 1 min at 700 × *g*.

Step	Action
2	**Equilibrate** • Add 400 µl binding buffer, mix briefly and centrifuge for 1 min at 700 × *g* to equilibrate the medium. • Perform this step 3 times total.
3	**Bind antibody** • Immediately after equilibration, add 200 µl of the antibody solution per well (0.5 to 1.0 mg/ml in binding buffer). • Incubate on shaker for 30 min. • Centrifuge for 1 min at 700 × *g* to remove unbound antibody.
4	**Wash** • Add 400 µl binding buffer and mix briefly. • Centrifuge for 1 min at 700 × *g*.
5	**Change buffer** • Add 400 µl triethanolamine and mix briefly. • Centrifuge for 1 min at 700 × *g*.
6	**Cross-link** • Add 400 µl DMP in triethanolamine. • Incubate on shaker for 30-60 min. • Centrifuge for 1 min at 700 × *g*.
7	**Wash** • Add 400 µl triethanolamine and mix briefly. • Centrifuge for 1 min at 700 × *g*.
8	**Block** • Add 400 µl ethanolamine • Incubate on shaker for 15 min. • Centrifuge for 1 min at 700 × *g*.
9	**Remove unbound antibody** • Add 400 µl elution buffer and mix briefly. • Centrifuge for 1 min at 700 × *g*.
10	**Wash** • Add 400 µl binding buffer, mix briefly and centrifuge for 1 min at 700 × *g*. • Perform this step 2 times total.
11	**Bind target protein** • Add 200 µl of sample in binding buffer. • Incubate on shaker for 60 min. • Replace the collection plate with a clean collection plate. • Centrifuge for 1 min at 700 × *g* to collection out unbound sample. • During optimization/trouble shooting: Collect flowthrough.
12	**Wash** • Replace the collection plate with a clean collection plate. • Add 400 µl collection buffer, mix briefly and centrifuge for 1 min at 700 × *g*. • Perform this step 5 times total.
13	**Elute** • Collect the eluates in separate collection plates. • Add 200 µl of elution buffer, mix briefly and centrifuge for 1 min at **1000 ×** *g*. • Perform this step three 3 times total.

Classic protocol

The protocol may need optimization for your application, see *Advice on handling, on page 3*. Please refer to *General handling of the MultiTrap plate, on page 1* for general handling instructions.

Step	Action
1	**Remove storage solution** • Suspend the medium by gently shaking the plate upside down. • Remove top and bottom seals and place the MultiTrap plate on a collection plate. • Remove the storage solution by centrifugation for 1 min at 700 x *g*.
2	**Equilibrate** • Add 400 µl binding buffer per well, mix briefly and centrifuge for 1 min at 700 × *g* to equilibrate the medium. • Perform this step 3 times total.
3	**Bind antibody** • Immediately after equilibration, add 200 µl of the antibody solution per well (0.5 to 1.0 mg/ml in binding buffer). • Incubate on shaker for 30 min. • Centrifuge for 1 min at 700 × g to remove unbound antibody.
4	**Wash** • Add 400 µl binding buffer per well and mix briefly. • Centrifuge for 1 min at 700 × *g*.
5	**Bind target protein** • Add 200 µl sample in binding buffer per well. • Incubate on shaker for 60 min. • Replace the collection plate with a clean collection plate. • Centrifuge for 1 min at 700 × *g* to wash out unbound sample. • During optimization/trouble shooting: Collect flowthrough.

Step	Action
6	**Wash** • Replace the collection plate with a clean collection plate. Collect and save washes in case troubleshooting is needed. • Add 400 µl wash buffer per well, mix briefly and centrifuge for 1 min at 700 × *g*. • Perform this step 5 times total.
7	**Elute** • Collect the eluates in separate collection plates. • Add 200 µl of desired elution buffer per well and shake for 1 min. • Centrifuge for 1 min at 700 x *g*. • Perform this procedure 3 times total.

Characteristics

Matrix	Highly cross-linked agarose, 6%
Medium	Protein G Sepharose High Performance
Ligand	Recombinant Protein G
Ligand coupling method	N-hydroxysuccinimide activation
Ligand density	approx. 2 mg Protein G/ml medium
Binding capacity[1]	approx. 25 mg human IgG/ml medium
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
Filter plate material	Polypropylene and polyethylene
Filter plate size[3]	127.8 x 85.5 x 30.6 mm
Volume, prepacked medium/well	50 µl
Well volume	800 µl
Centrifugation speed[4]	700 x *g*
Vacuum pressure[4]	
• Recommended	-0.1 to -0.3 bar
• Maximum	-0.5 bar

[1] Protein dependent

[2] pH below 3 is sometimes required to elute strongly bound Ig species. However, protein ligands may hydrolyze at very low pH.

[3] According to American Standard Institute (ANSI) and Society for Biomolecular Screening (SBS) standards 1-2004, 3-2004 and 4-2004.

[4] Actual settings will depend on the sample properties and pretreatment.

Ordering information

Products

Description	Quantity	Product code
Protein G HP MultiTrap	4 x 96-well filter plates	28903135

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 x 96-well filter plates	28903131
Protein A HP SpinTrap	16 columns	28903132
Protein A HP MultiTrap	4 x 96-well filter plates	28903133
Protein G HP SpinTrap	16 columns	28903134
Collection Plate	5 x 96 well plates	28403943
Ab SpinTrap	50 x 100 µl	28408347
Ab Buffer Kit	1	28903059
Protein A/G SpinTrap Buffer Kit	1	28913567

Literature

Title	Product code
Data File Protein G HP	28906790
Antibody Purification Handbook	18103746
Affinity Chromatography Handbook	18102229

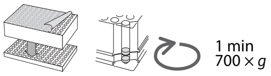
Antibody purification quick protocol

1. Prepare collection plates

prepare 2 collection plates for eluted fractions, each containing 15 µl neutralizing buffer per well.

2. Remove storage solution

- Shake gently upside down
- Remove the seals



3. Equilibrate

- Add 300 µl binding buffer

4. Bind antibody

- Add 300 µl antibody in binding buffer
- Incubate 4 min on shaker

5. Wash

- Add 300 µl binding buffer

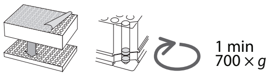
6. Elute antibody

- Add 200 µl binding buffer

Cross-link quick protocol

1. Remove storage solution

- Shake gently upside down
- Remove the seals



2. Equilibrate

- Add 400 µl binding buffer

3 × 1 min 700 × *g*

3. Bind antibody

- Add 200 µl antibody in binding buffer
- Incubate 30 min on shaker

1 min 700 × *g*

4. Wash

- Add 400 µl binding buffer

1 min 700 × *g*

5. Change buffer

- Add 400 µl triethanolamine

1 min 700 × *g*

6. Cross-link

- Add 400 µl DMP in triethanolamine
- Incubate 60 min on shaker

1 min 700 × *g*

7. Wash

- Add 400 µl triethanolamine

1 min 700 × *g*

8. Block

- Add 400 µl ethanolamine
- Incubate 15 min on shaker

1 min 700 × *g*

9. Remove unbound antibody

- Add 400 µl elution buffer

2 × 1 min 700 × *g*

10. Wash

- Add 400 µl binding buffer

2 × 1 min 700 × *g*

11. Bind target protein

- Add 200 µl sample in binding buffer
- Incubate 60 min on shaker

1 min 700 × *g*

12. Wash

- Add 400 µl wash buffer

5 × 1 min 700 × *g*

13. Elute

- Add 200 µl elution buffer
- Shake for 1 min

3 × 1 min 1000 × *g*

Classic quick protocol

1. Remove storage solution

- Shake gently upside down
- Remove the seals

1 min 700 × *g*

2. Equilibrate

- Add 400 µl binding buffer

3 × 1 min 700 × *g*

3. Bind antibody

- Add 200 µl antibody in binding buffer
- Incubate 30 min on shaker

1 min 700 × *g*

4. Wash

- Add 400 µl binding buffer

1 min 700 × *g*

5. Bind target protein

- Add 200 µl sample in binding buffer
- Incubate 60 min on shaker

1 min 700 × *g*

6. Wash

- Add 400 µl wash buffer

5 × 1 min 700 × *g*

7. Elute

- Add 200 µl elution buffer
- Shake for 1 min

3 × 1 min 700 × *g*

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

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