cytiva

Protein A Mag **Sepharose** Xtra
Protein G Mag **Sepharose** Xtra

Affinity chromatography

Instructions for Use

cytiva.com

28967057 AB

Abstract

Protein A Mag Sepharose™ Xtra and Protein G Mag Sepharose Xtra are available in the following pack sizes (Instructions for use included in all pack sizes):

- Protein A Mag Sepharose Xtra, 10% medium slurry, 2 × 1 ml
- Protein A Mag Sepharose Xtra, 10% medium slurry, 5 × 1 ml
- Protein G Mag Sepharose Xtra, 10% medium slurry, 2 × 1 ml
- Protein G Mag Sepharose Xtra, 10% medium slurry, 5 × 1 ml

Note: *1 ml medium slurry is sufficient for 10 reactions according to the recommended protocol. 1 ml of 10% (v/v) medium slurry contains 100 µl magnetic beads.*

Purpose
Protein A Mag Sepharose Xtra and Protein G Mag Sepharose Xtra products are magnetic beads designed for high capacity small-scale purification/screening of monoclonal and polyclonal antibodies from various species.

Intended use
Protein A Mag Sepharose Xtra and Protein G Mag Sepharose Xtra are intended for research only, and should not be used in any clinical or *in vitro* procedures for diagnostic purposes.

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1 Principle

Protein A Mag Sepharose Xtra and Protein G Mag Sepharose Xtra are affinity chromatography media with high affinity for antibodies from various species. The media are designed for high capacity, which makes them useful for efficient small-scale purification/screening of monoclonal and polyclonal antibodies. The products are magnetic beads based on Sepharose coupled with protein A or protein G ligands.

Protein A Mag Sepharose Xtra and Protein G Mag Sepharose Xtra provide flexible purification allowing a wide range of sample volumes and easy scaling up by varying the bead quantity.

Mag Sepharose products can be used together with Eppendorf microcentrifuge tubes and a magnetic rack, for example MagRack 6 (see Section 3 Advice on handling). The magnetic beads are easily separated from the liquid phase during the different steps of the purification protocol.

Note: *For immunoprecipitation, it is recommended to use the corresponding products Protein A Mag Sepharose and Protein G Mag Sepharose (see Section 8 Ordering Information). These products have optimized capacities for immunoprecipitation applications.*

2 Antibody binding to protein A and protein G

The binding strengths of protein A and protein G for immunoglobulins depend on the source species and subclass of the particular immunoglobulin.

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	+	++++
	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

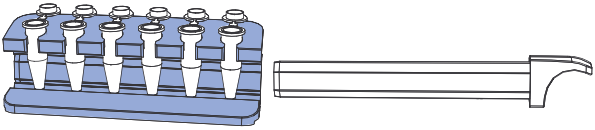
3 Advice on handling

Note: *Protein A Mag Sepharose Xtra and Protein G Mag Sepharose Xtra are intended for single use only.*

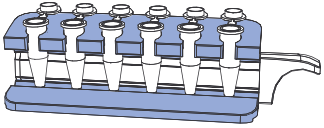
General magnetic separation step

It is recommended to use 1.5 ml Eppendorf tubes and MagRack 6 in the included protocol (see Section 5).

1 *Remove* the magnet before *adding* liquid.



2 *Insert* the magnet before *removing* liquid.



When using volumes above 1.5 ml, e.g. 50 ml, a magnetic pickpen can be used for collecting the magnetic beads. Another alternative is to spin down the beads by using a swing-out centrifuge.

Dispensing the medium slurry

- Prior to dispensing the medium slurry, make sure it is homogeneous by vortexing.
- When the medium slurry is resuspended, pipette *immediately* the required amount of medium slurry into the desired tube.
- Repeat the resuspension step between each pipetting from the medium slurry vial.

Handling of liquid

- Use the magnetic rack with the magnet in place for each liquid removal step.
- Before application of liquid, remove the magnet from the magnetic rack.
- After addition of liquid, allow resuspension of the beads by vortexing or manual inversion of the tube. When processing multiple samples, manual inversion of the magnetic rack is recommended.

Incubation steps

- During incubation steps, make sure the magnetic beads are well resuspended and kept in solution by end-over-end mixing or by using a benchtop shaker.
- Incubation steps generally take place at room temperature. However, incubation can take place at +4°C over night if this is the recommended storage condition for the specific sample.
- When purifying samples of low concentrations or large volumes, an increase of the incubation time might be necessary.
- If needed, a pipette can be used to remove liquid from the lid.

4 Operation

Recommended buffers

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

Table 2. Recommended buffers.

Buffer	Composition
Binding buffer	• PBS (137 mM NaCl, 2.7 mM KCl, 100 mM Na_2HPO_4, 2 mM KH_2PO_4), pH 7.4
Elution buffer	• 100 mM glycine-HCl, pH 2.8

- Protein A Mag Sepharose Xtra and Protein G Mag Sepharose Xtra bind immunoglobulins over a wide pH range and thus permits the use of a variety of buffers. Generally, Protein A Mag Sepharose Xtra and Protein G Mag Sepharose Xtra bind IgG with a strong affinity at pH 7.
- Different immunoglobulins elute at different pH values depending on subclass and the species from which they originate. For antibodies sensitive to low pH, optimize elution by determining the highest pH that allows efficient elution.
- Suitable buffers can also be easily prepared using Ab Buffer Kit (see Section 8).

Sample pretreatment

- Check the pH of the sample, and adjust if necessary before applying the sample to the beads. The pH of the sample should equal the pH of the binding buffer. Adjusting the pH could be done by either diluting the sample with binding buffer or by buffer exchange using PD MiniTrap™ G-25 or HiTrap™ Desalting.
- Clarification of sample might be needed before applying it to the beads.

5 Antibody purification protocol

This protcol is suitable for most antibody purifications.

1 Magnetic bead preparation

A Mix the medium slurry thoroughly by vortexing. Dispense 100 µl homogenous medium slurry into an Eppendorf tube.

B Place the Eppendorf tube in the magnetic rack, for example MagRack 6 (see Section 3).

C Remove the storage solution.

2 Equilibration

A Add 500 µl binding buffer.

B Resuspend the medium.

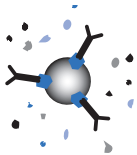
C Remove the liquid.

3 Sample application

A Immediately after equilibration, add 300 µl of sample. If the sample volume is less than 300 µl, dilute to 300 µl with binding buffer.

B Resuspend the medium and incubate for 30 minutes with slow end-over-end mixing or by using a benchtop shaker.

C Remove the liquid.



4 Washing (perform this step 2 times totally)

A Add 500 µl binding buffer.

B Resuspend the medium.

C Remove the liquid.

5 Elution

A Add 100 µl of elution buffer.

B Resuspend the medium.

C Remove and collect the elution fraction. The collected elution fraction contains the main part of the purified antibody. If desired, repeat the elution.

Note: *As a safety measure to preserve the activity of acid-labile antibodies, we recommend the addition of 1 M Tris-HCl, pH 9.0, to tubes used for collecting antibody-containing fractions.*

6 Optimization of parameters

The protocol recommended in this instruction (see Section 5) is suitable for purification of most antibodies. However, some parameters for antibody purification may require optimization to obtain the best result.

Examples of parameters which may require optimization are:

- Amount of beads
- Incubation times
- Choice of buffers
- Number of washes

7 Characteristics

Table 3. Protein A Mag Sepharose Xtra.

Matrix	Highly crosslinked spherical agarose (Sepharose) including magnetite
Medium	Protein A coupled NHS activated Mag Sepharose
Ligand	Protein A
Binding capacity	>27 mg human IgG/ml gel
Particle size	37 to 100 µm
Working temperature	Room temperature
Storage solution	20% ethanol, 10% medium slurry
Storage temperature	+4°C to +8°C

Table 4. Protein G Mag Sepharose Xtra.

Matrix	Highly crosslinked spherical agarose (Sepharose) including magnetite
Medium	Protein G coupled NHS activated Mag Sepharose
Ligand	Protein G
Binding capacity	>27 mg human IgG/ml gel
Particle size	37 to 100 µm
Working temperature	Room temperature
Storage solution	20% ethanol, 10% medium slurry
Storage temperature	+4°C to +8°C

8 Ordering Information

Products	Quantity	Product code
Protein A Mag Sepharose Xtra	2 × 1 ml 10% medium slurry	28-9670-56
Protein A Mag Sepharose Xtra	5 × 1 ml 10% medium slurry	28-9670-62
Protein G Mag Sepharose Xtra	2 × 1 ml 10% medium slurry	28-9670-66
Protein G Mag Sepharose Xtra	5 × 1 ml 10% medium slurry	28-9670-70

Related products	Quantity	Product code
MagRack 6	1	28-9489-64
Ab Buffer Kit	1	28-9030-59
HiTrap Desalting	5 × 5 ml	17-1408-01
PD MiniTrap G-25	50 columns	28-9180-07
His Mag Sepharose Ni	2 × 1 ml 5% medium slurry	28-9673-88
His Mag Sepharose Ni	5 × 1 ml 5% medium slurry	28-9673-90
Protein A Mag Sepharose	1 × 500 µl 20% medium slurry	28-9440-06
Protein A Mag Sepharose	4 × 500 µl 20% medium slurry	28-9513-78
Protein G Mag Sepharose	1 × 500 µl 20% medium slurry	28-9440-08
Protein G Mag Sepharose	4 × 500 µl 20% medium slurry	28-9513-79
NHS Mag Sepharose	1 × 500 µl 20% medium slurry	28-9440-09
NHS Mag Sepharose	4 × 500 µl 20% medium slurry	28-9513-80
TiO_2 Mag Sepharose	1 × 500 µl 20% medium slurry	28-9440-10
TiO_2 Mag Sepharose	4 × 500 µl 20% medium slurry	28-9513-77

Antibody purification protocol

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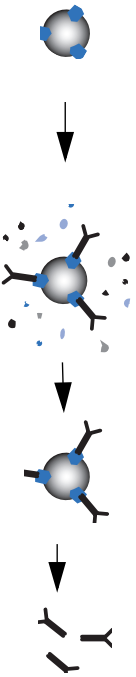

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