

User Guide

PureProteome™ Kappa and Lambda Ig Binder Magnetic Beads

For Human Immunoglobulins Only

LSKMAGKP02, LSKMAGLM02

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for human or animal consumption.

Introduction

PureProteome™ Kappa and Lambda Ig Binder Magnetic beads have been developed using an antibody ligand specific for human kappa light chain (subclass I, II, III, and IV) or lambda light chain (subclass I, II, III, and IV) capable of capturing all immunoglobulin subtypes (IgG, IgA, IgD, IgE, and IgM). These magnetic beads provide a rapid, scalable, and reproducible means to capture human antibody or antibody fragments containing kappa or lambda light chains, including Fab and F(ab')₂. The Kappa Magnetic Beads bind to the kappa light chain constant region on human immunoglobulins with high specificity, and the Lambda Magnetic Beads bind to the lambda light chain constant region on human immunoglobulins with high specificity.

Depletion of all human immunoglobulins can be performed by mixing PureProteome™ Kappa and Lambda Magnetic beads.

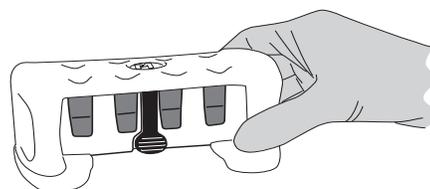
Components

PureProteome™ Kappa and Lambda Ig Binder Magnetic Beads

	Catalogue No.
Kappa Magnetic Beads, 2 mL of 10% slurry (10 mg/mL)	LSKMAGKP02
Lambda Magnetic Beads, 2 mL of 10% slurry (10 mg/mL)	LSKMAGLM02

Materials Required but Not Supplied

- 1.5 mL microcentrifuge tubes
- Recommended Buffers:
 - Binding Buffer: Phosphate-buffered saline (PBS) or Tris-buffered saline (TBS)
 - Wash Buffer: PBS, TBS, or Tris-buffered saline with Tween® 20 detergent (TBS-T)
 - Elution Buffer: 200 mM Glycine-HCl, pH 2.0–3.0 or 100 mM Triethylamine, pH 11.5
 - Neutralizing Buffer: 2 M Tris, pH 8.0 (optional) or 2 M Tris, pH 7.0 (optional)
- PureProteome™ Magnetic Stand (8-well), LSKMAGS08



For optimal performance, the PureProteome™ Magnetic Stand is recommended for use with PureProteome™ Kappa and Lambda Magnetic Beads.

General Procedure for Using PureProteome™ Kappa and Lambda Ig Binder Magnetic Beads

The user will need to optimize the capture, wash, and elution conditions for the antibody or antibody fragment of interest. Depending on the application, the user may need to use kappa only, or lambda only, or a mix of both kappa and lambda beads.

1. Calculate the volume of bead slurry required to bind the desired amount of target.
2. Mix the bead slurry so that all of the beads are uniformly resuspended. To ensure consistent bead volume, mix immediately before pipetting each aliquot.
3. Pipette the desired volume of resuspended bead slurry into a microcentrifuge tube. Place the tube into the PureProteome™ Magnetic Stand and allow the beads to migrate to the magnet. Remove the storage buffer with a pipette and discard.
4. Wash the beads twice using 500 μ L of wash buffer for each wash. Disengage the magnet from the stand and vortex vigorously for 10 seconds. Re-engage the magnet and allow the beads to migrate to the magnet. Remove the buffer with a pipette and discard.
5. Add the sample to the beads. Ensure that the sample volume is at least 5 times the settled bead volume. Incubate for 45–75 minutes at room temperature with continuous mixing or end-over-end rotation.
6. A pulse in a microcentrifuge may be required if liquid has accumulated in the microcentrifuge tube cap. Place the tube back into the magnetic stand. Allow the beads to migrate to the magnet. Remove the depleted sample with a pipette. Transfer to a fresh tube and save if optimizing the protocol.
7. Wash the beads 3 times using 500 μ L of wash buffer for each wash. After each wash, disengage the magnet and vortex vigorously for 10 seconds. Re-engage the magnet and allow the beads to migrate to the magnet. Remove the buffer with a pipette and discard. Wash fractions may be saved if optimizing the protocol.
8. Resuspend the beads in 5–10 volumes of elution buffer and incubate with continuous mixing or end-over-end rotation for 5–10 minutes.
9. A pulse in a microcentrifuge may be required if liquid has accumulated in the microcentrifuge tube cap. Place the tube back into the magnetic stand. Allow the beads to migrate to the magnet. Remove the eluted sample with a pipette. Transfer to a fresh tube and save. Repeat the elution step twice more to ensure recovery of all bound sample.
NOTE: Samples eluted in 200 mM Glycine-HCl, pH 2.0-3.0 may need to be neutralized by adding 1/10 the eluted sample volume of 2M Tris, pH 8.0.
Samples eluted in 100 mM Triethylamine, pH 11.5 may need to be neutralized by adding 1/10 the eluted sample volume of 2 M Tris, pH 7.0.
The input, unbound, and bound (eluted) fraction of proteins may be analyzed by SDS-PAGE to ensure recovery of target protein.
10. Optional: The beads may be further eluted in 1X SDS-PAGE Reducing Sample Buffer (RSB) to ensure recovery of all bound sample. Resuspend the beads in a minimum of 100 μ L of 1X RSB. After adding the RSB, disengage the magnet and vortex vigorously for 10 seconds. Remove tube and incubate at 70 °C for 10 minutes. Quickly place the tube back in the magnetic stand and allow the beads to migrate to the magnet. Immediately remove the eluted fraction with a pipette and save. Repeat two more times.
NOTE: The antibody ligand (~13 MW) may be observed on the gel if the magnetic beads have been incubated in 1X Reducing Sample Loading Buffer (RSB) at 70 °C for 10 minutes.

Immunoglobulin Depletion with PureProteome™ Kappa and Lambda Ig Binder Magnetic Beads

This method is intended for the immunoglobulin depletion from 25 μ L of human serum. Optimization may be required and will depend on the end user application and sample composition.

1. Mix the Kappa and Lambda Magnetic Bead slurry immediately before use to uniformly suspend magnetic beads.
2. Pipette 150 μ L of each resuspended bead slurry into a microcentrifuge tube.
3. Place the tube into the PureProteome™ Magnetic Stand and allow the beads to migrate to the magnet. Remove the storage buffer with a pipette and discard.
4. Wash the beads twice using 500 μ L of binding buffer for each wash. Disengage the magnet from the stand and vortex vigorously for 10 seconds. Re-engage the magnet and allow the beads to migrate to the magnet. Remove the buffer with a pipette and discard. A pulse in a microcentrifuge may be required if liquid has accumulated in the microcentrifuge tube cap.
5. Dilute 25 μ L of human serum to a final volume of 100 μ L with binding buffer.
6. Add the diluted serum sample to the magnetic beads. Incubate for 60 minutes with continuous mixing or end-over end-rotation.
7. Place the tube into the magnetic stand, re-engage the magnet, and collect the magnetic beads. Remove the depleted sample with a pipette. Transfer to a fresh tube and save.
8. To increase sample recovery, beads can be washed 3 times with 150 μ L binding buffer; the majority of residual protein will be recovered in the first wash step.
9. If desired, the wash buffer fraction/fractions may be combined with the depleted sample. The combined sample can be further concentrated and buffer-exchanged using Amicon® Ultra centrifugal devices.
10. Optional: Immunoglobulin elution can be facilitated by resuspending the beads in 150–200 μ L elution buffer and incubating with continuous mixing or end-over-end mixing for 5–10 minutes.
11. A pulse in a microcentrifuge may be required if liquid has accumulated in the microcentrifuge tube cap. Place the tube back into the magnetic stand. Allow the beads to migrate to the magnet. Remove the eluted sample with a pipette. Transfer to a fresh tube and save. Repeat the elution step twice more to ensure recovery of all bound sample.

NOTE: Samples eluted in 200 mM Glycine-HCl, pH 2.0-3.0 may need to be neutralized by adding 1/10 the eluted sample volume of 2M Tris, pH 8.0.

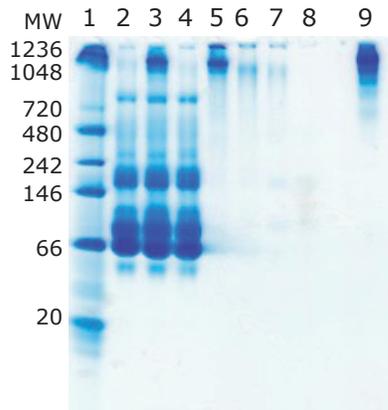
Samples eluted in 100 mM Triethylamine, pH 11.5 may need to be neutralized by adding 1/10 the eluted sample volume of 2 M Tris, pH 7.0.

Performance

	IgA	IgG ₁	IgG ₂	IgG ₃	IgG ₄	IgD	IgE	IgM
(%)	99.79	99.94	99.91	99.98	99.83	99.99	99.11	99.92
Depletion								

Table 1: Efficiency of Immunoglobulin Capture from Human Serum.

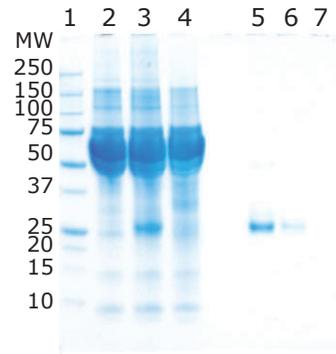
A blend of 150 µL each of Kappa Magnetic Bead slurry and Lambda Magnetic Bead slurry was used to capture (deplete) immunoglobulins from 25 µL human serum (pooled). The % Ig captured was determined by measuring immunoglobulins in the input and unbound serum samples by ELISA (IgD and IgE) and Luminex® assay (IgA, IgG₁, IgG₂, IgG₃, IgG₄, and IgM).



- Lane 1 Markers
- Lane 2 Hybridoma media
- Lane 3 Hybridoma media spiked with human IgM (input)
- Lane 4 Unbound fraction
- Lane 5 Eluate fraction 1
- Lane 6 Eluate fraction 2
- Lane 7 Eluate fraction 3
- Lane 8 Eluate fraction 4
- Lane 9 Purified human IgM

Figure 1: Non-denatured PAGE of Fractions of Human IgM Purification from Hybridoma Media

100 µg of purified human kappa IgM was spiked into 100 µL of hybridoma media. 250 µL of Kappa Magnetic Bead slurry was incubated with the spiked hybridoma media for 1 hour with continuous mixing. Beads were washed with PBS and eluted four times with 50 µL of 100 mM Triethylamine, pH 11.5. 5 µL of each fraction was resolved on a non-denatured gel and Coomassie stained.



- Lane 1 Markers
- Lane 2 Hybridoma media
- Lane 3 Hybridoma media spiked with human lambda Fab (input)
- Lane 4 Unbound fraction
- Lane 5 Eluate fraction 1
- Lane 6 Eluate fraction 2
- Lane 7 Eluate fraction 3

Figure 2: SDS-PAGE of Fractions of Human Lambda Fab Purification from Hybridoma Media

100 µL of Lambda Magnetic Bead slurry was used to capture the Fab fragment from 100 µL of hybridoma media spiked with human lambda Fab. The captured protein was eluted 3 times using 100 µL of 100 mM Triethylamine, pH 11.5. 10 µL of each fraction was resolved by SDS-PAGE and Coomassie stained.

Specifications

	PureProteome™ Kappa Ig Binder Magnetic Beads	PureProteome™ Lambda Ig Binder Magnetic Beads
Presentation	10% (v/v) slurry in 10 mM Tris-HCl and 0.05% sodium azide, pH 8.0 ±0.2	10% (v/v) slurry in 10 mM Tris-HCl and 0.05% sodium azide, pH 8.0 ±0.2
Matrix	Antibody ligand covalently coupled to magnetic silica-based beads	Antibody ligand covalently coupled to magnetic silica-based beads
Particle form	Spherical	Spherical
Bead diameter	10 µm (nominal)	10 µm (nominal)
Storage	2–8 °C, do not freeze	2–8 °C, do not freeze
Capacity	≥ 2.2 µg kappa IgG per µL slurry	≥ 2.0 µg lambda IgG per µL slurry
Shelf life	12 months from date of receipt	Refer to expiration date on product label

PureProteome™ Kappa and Lambda Ig Binder Magnetic Beads are for research use only. They are not for use in diagnostic procedures.

Disposal

Collect and dispose of used material according to all applicable international, federal, state, and local regulations.

Safety Data Sheet

Safety Data Sheets (SDS) are available on our web site. Go to [SigmaAldrich.com](https://www.sigmaaldrich.com) and enter your catalogue number in the search box.

Product Ordering

Description	Qty/Pk	Catalogue No.	Description	Qty/Pk	Catalogue No.
PureProteome™ Kappa Ig Binder Magnetic Beads	2 mL	LSKMAGKP02	PureProteome™ Streptavidin Magnetic Beads	2 × 1 mL 1 × 10 mL	LSKMAGT02 LSKMAGT10
PureProteome™ Lambda Ig Binder Magnetic Beads	2 mL	LSKMAGLM02	PureProteome™ Nickel Magnetic Beads	2 × 1 mL 1 × 10 mL	LSKMAGH02 LSKMAGH10
PureProteome™ Human Albumin/Immunoglobulin Depletion Kit	1	LSKMAGHDKIT	PureProteome™ Protein A Magnetic Beads	2 × 1 mL 1 × 10 mL	LSKMAGA02 LSKMAGA10
Contains magnetic beads, buffer concentrate, and Amicon® Ultra-2 3K devices			PureProteome™ Protein G Magnetic Beads	2 × 1 mL 1 × 10 mL	LSKMAGG02 LSKMAGG10
PureProteome™ Albumin Magnetic Beads	1 × 10 mL	LSKMAGL10	PureProteome™ NHS FlexiBind Magnetic Beads Kit	1	LSKMAGN01
PureProteome™ Albumin/IgG Depletion Kit	1	LSKMAGD12	Contains 0.5 mL magnetic beads, equilibration, wash/coupling, and quench buffers, and Amicon® Ultra-0.5 devices		
Contains magnetic beads, buffer concentrate, and Amicon® Ultra-4 devices			PureProteome™ NHS FlexiBind Magnetic Beads	4 × 0.5 mL	LSKMAGN04
PureProteome™ Magnetic Stand, 8-well	1	LSKMAGS08	PureProteome™ Carboxy Flexibind Magnetic Beads		
PureProteome™ Magnetic Stand, 15 mL	1	LSKMAGS15	0.3 µm beads	2 mL 10 mL	LSKMAG03CBX02 LSKMAG03CBX10
Amicon® Ultra-2 3K Device	24	UFC200324	1.0 µm beads	2 mL 10 mL	LSKMAG1CBX02 LSKMAG1CBX10
			2.5 µm beads	2 mL 10 mL	LSKMAG25CBX02 LSKMAG25CBX10

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