User Guide

PureProteome™ Carboxy FlexiBind Magnetic Beads

LSKMAG03CBX02, LSKMAG03CBX10, LSKMAG1CBX02, LSKMAG1CBX10, LSKMAG25CBX02, LSKMAG25CBX10

FOR RESEARCH USE ONLY

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

Introduction

PureProteome™ Carboxy Flexibind Magnetic Beads offer the flexibility to permanently bind and immobilize a variety of amine-containing ligands. The high density of carboxylic acid groups (COOH) on the surface of the beads can be used for covalent coupling of ligands such as proteins, peptides, and nucleic acids. The carboxyl groups on the surface of the beads are activated with EDC, allowing the ligand to be coupled to the beads through primary amine groups, forming a stable amide bond.

PureProteome™ Carboxy FlexiBind Magnetic Beads are uniform, polystyrene-based, superparamagnetic beads amenable for use in a variety of magnetic separation applications including affinity enrichment, protein purification, immunoprecipitation, and cell depletion. Carboxy FlexiBind Magnetic Beads are available in three sizes; 0.3 µm, 1.0 µm, and 2.5 µm.

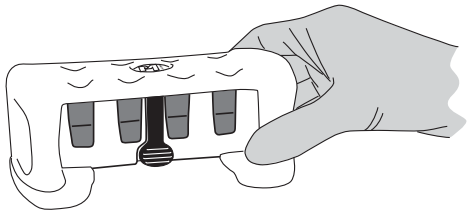
Components

PureProteome™ Carboxy FlexiBind Magnetic Bead	Catalogue No.
0.3 µm bead, 2 mL of 1% slurry (10 mg/mL)	LSKMAG03CBX02
0.3 µm bead, 10 mL of 1% slurry (10 mg/mL)	LSKMAG03CBX10
1.0 µm bead, 2 mL of 1% slurry (10 mg/mL)	LSKMAG1CBX02
1.0 µm bead, 10 mL of 1% slurry (10 mg/mL)	LSKMAG1CBX10
2.5 µm bead, 2 mL of 3% slurry (30 mg/mL)	LSKMAG25CBX02
2.5 µm bead, 10 mL of 3% slurry (30 mg/mL)	LSKMAG25CBX10

MERCK

Materials Required but Not Supplied

- 1.5 mL microcentrifuge tubes
- Activation/Coupling Buffer: 50 mM MES [2-(N-morpholino)-ethanesulfonic acid], pH 6.0, 0.01% Triton® X-100 detergent
- Coupling Agent: EDC [1-ethyl-3-(3-dimethyaminopropyl)-carbodiimide]
- Quench Buffer: TBS (25 mM Tris-Cl, 130 mM NaCL, 2.7 mM KCl), pH 8, 0.01% Triton® X-100 detergent
- Storage Buffer: TBS or PBS containing 0.01% Triton® X-100 detergent or 0.01% Tween® 20 detergent, add preservative if needed
- Sulfo-NHS [N-hydroxysulfosuccinimide] (Optional)
- PureProteome™ Magnetic Stand, 8-well, (LSKMAGS08)



For optimal performance, the PureProteome™ Magnetic Stand is recommended for use with PureProteome™ Carboxy FlexiBind Magnetic Beads.

Procedure for Using PureProteome™ Carboxy FlexiBind Magnetic Beads

The following protocol provides general guidelines for the coupling of ligands to 100 µL of PureProteome™ Carboxy FlexiBind magnetic bead slurry. The protocol may be scaled up or down as desired. Optimization of bead activation (EDC, EDC/NHS, and pH), and coupling conditions (ligand concentration, coupling buffer, pH, reaction volume, and incubation time) for the ligand of interest are strongly recommended.

NOTES:

- Ensure that the magnetic beads are uniformly resuspended by vortexing the tube.
- For best performance, proteins should be at a concentration of 0.5–4 mg/mL and oligonucleotides at 20–100 µM in an amine-free coupling buffer, free of other proteins. A higher concentration of protein allows flexibility in optimizing the reaction volume. Buffers containing tris, glycine, acetate, or citrate cannot be used.
- 50 mM MES buffer, pH 6.0, containing 0.01% Triton® X-100 detergent, can be used as both the activation and coupling buffer. When using chemically synthesized oligonucleotides, a 5’ amine group should be included in the synthesis for coupling to the beads.
- The beads contain 0.5% SDS to stabilize the suspension. Although not required, inclusion of 0.01–0.05% Triton® X-100 detergent or 0.01–0.05% Tween® 20 detergent in all buffers will prevent bead clumping and improve handling.

General Coupling Protocol A Two-Step Coupling with EDC

This protocol is recommended for conjugating protein to the beads. The carboxyl groups on the beads are first activated using a water soluble carbodiimide (EDC) to form an amine-reactive intermediate. After removing EDC, the protein ligand is added and coupled to the beads through primary amines on the protein.

1. Ensure that the protein or ligand is in an amine-free coupling buffer. 50–400 µg of protein in 100 µL coupling buffer is required for conjugation. Place on ice.
2. Resuspend the PureProteome™ Carboxy Magnetic Bead slurry by vortexing.
3. Pipet 100 µL of bead slurry into a 1.5 mL microcentrifuge tube.
4. 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.
5. Disengage the magnet and add 200 µL of activation/coupling buffer. Vortex vigorously for 20 seconds. Re-engage the magnet and allow the beads to migrate to the magnet. Remove the buffer with a pipette and discard.
6. Wash the beads two more times as in step 5.
7. Immediately before use prepare EDC (50 mg/mL) in activation/coupling buffer.
8. Disengage the magnet from the magnetic stand and add 80 µL of activation/coupling buffer and 20 µL of the freshly prepared EDC solution to the beads. Vortex to mix well.
9. Incubate beads with continuous mixing for 15 minutes at room temperature.
10. A pulse in a microcentrifuge may be required if liquid has collected in the microcentrifuge tube cap. Place tube into magnetic stand. Re-engage the magnet and allow the beads to migrate to the magnet. Remove the supernatant with a pipette and discard.
11. Disengage the magnet and wash the beads with 200 µL of activation/coupling buffer. Vortex to mix well, then re-engage the magnet and allow the beads to migrate to the magnet. Remove the supernatant with a pipette and discard.

    Note: This step should be performed QUICKLY, as the amine-reactive intermediate on the beads is unstable.
12. Disengage the magnet from the magnetic stand and add 50–400 µg protein or ligand in 100 µL activation/coupling buffer. Vortex to mix well.
13. Incubate beads with continuous mixing for 30 minutes at room temperature. Optimum incubation time may range from 0.5–4 hours, depending on ligand and concentration.
14. A pulse in a microcentrifuge may be required if liquid has collected in the microcentrifuge tube cap. Place microcentrifuge tube into the magnetic stand. Re-engage the magnet and allow the beads to migrate to the magnet. Remove the supernatant with a pipette. This supernatant contains the unbound ligand fraction and can be saved for analysis if optimizing the protocol.
15. Disengage the magnet and wash the beads with 100 µL of activation/coupling buffer. Vortex to mix well, then re-engage the magnet and allow the beads to migrate to the magnet. Remove the supernatant with a pipette and discard. This wash can be saved if optimizing the protocol.
16. Disengage the magnet and add 500 µL of quench buffer (TBS, pH 8, 0.01% Triton® X-100 detergent) to the beads. Vortex vigorously for 20 seconds. Re-engage the magnet and allow the beads to migrate to the magnet. Remove the buffer with a pipette and discard.
17. Disengage the magnet and add 500 µL of quench buffer (TBS, pH 8, 0.01% Triton® X-100 detergent). Incubate for 30–60 minutes at room temperature. Re-engage the magnet and allow the beads to migrate to the magnet. Remove the buffer with a pipette and discard.
18. Disengage the magnet and add 500 µL of quench buffer (TBS, pH 8, 0.01% Triton® X-100 detergent). Vortex vigorously for 20 seconds. Re-engage the magnet and allow the beads to migrate to the magnet. Remove the buffer with a pipette and discard.
19. Wash the beads twice more as in step 18.
20. Remove the microcentrifuge tube from the magnetic stand. Add 100 µL storage buffer. Vortex to mix well and store the beads at 2–8 °C.

General Coupling Protocol B Alternative Two-Step Coupling with Sulfo-NHS

This protocol is similar to the one above, but uses NHS. The efficiency of the EDC-mediated reaction may be increased by the addition of Sulfo-NHS which stabilizes the amine-reactive intermediate.

1. Ensure that the protein or ligand is in an amine-free coupling buffer. 50–400 µg of protein in 100 µL coupling buffer is required for conjugation. Place on ice.
2. Resuspend the PureProteome™ Carboxy Magnetic Bead slurry by vortexing.
3. Pipet 100 µL of bead slurry into a 1.5 mL microcentrifuge tube.
4. 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.
5. Disengage the magnet and add 200 µL of activation/coupling buffer. Vortex vigorously for 20 seconds. Re-engage the magnet and allow the beads to migrate to the magnet. Remove the buffer with a pipette and discard.
6. Wash the beads two more times as in step 5.
7. Immediately before use prepare EDC (50 mg/mL) in activation/coupling buffer.
8. Immediately before use prepare Sulfo-NHS (50 mg/mL) in activation/coupling buffer.
9. Disengage the magnet from the magnetic stand and add 60 µL of activation/coupling buffer, 20 µL of the freshly prepared EDC solution, and 20 µL of the freshly prepared Sulfo-NHS solution to the beads. Vortex to mix well.
10. Incubate beads with continuous mixing for 15 minutes at room temperature.
11. A pulse in a microcentrifuge may be required if liquid has collected in the microcentrifuge tube cap. Place tube into magnetic stand and allow the beads to migrate to the magnet. Remove the supernatant with a pipette and discard.
12. Disengage the magnet and wash the beads with 200 µL of activation/coupling buffer. Vortex to mix well. Re-engage the magnet and allow the beads to migrate to the magnet. Remove the supernatant with a pipette and discard.
13. Disengage the magnet from the magnetic stand. Add 50–400 µg protein or ligand in 100 µL activation/coupling buffer. Vortex to mix well.
14. Incubate beads with continuous mixing for 0.5–4 hours at room temperature.
15. A pulse in a microcentrifuge may be required if liquid has collected in the microcentrifuge tube cap. Place tube into magnetic stand and allow the beads to migrate to the magnet. Remove the supernatant with a pipette. This supernatant contains the unbound ligand fraction and can be saved for analysis if optimizing the protocol.
16. Disengage the magnet and wash the beads with 100 µL of activation/coupling buffer. Vortex to mix well, then re-engage the magnet and allow the beads to migrate to the magnet. Remove the supernatant with a pipette and discard. This wash can be saved if optimizing the protocol.
17. Disengage the magnet and add 500 µL of quench buffer (TBS, pH 8, 0.01% Triton® X-100 detergent) to the beads. Vortex vigorously for 20 seconds. Re-engage the magnet and allow the beads to migrate to the magnet. Remove the buffer with a pipette and discard.
18. Disengage the magnet and add 500 µL of quench buffer (TBS, pH 8, 0.01% Triton® X-100 detergent). Incubate for 30–60 minutes at room temperature. Re-engage the magnet and allow the beads to migrate to the magnet. Remove the buffer with a pipette and discard.
19. Disengage the magnet and add 500 µL of quench buffer (TBS, 0.01% Triton® X-100 detergent). Vortex vigorously for 20 seconds. Re-engage the magnet and allow the beads to migrate to the magnet. Remove the buffer with a pipette and discard.
20. Wash the beads two more times as in step 19.
21. Remove the microcentrifuge tube from the magnetic stand. Add 100 µL storage buffer. Vortex to mix well and store the beads at 2–8 °C.

General Coupling Protocol C One-Step Coupling

This is a quick conjugation protocol, combining beads, EDC, and ligand in one reaction. This protocol is best suited for coupling oligonucleotides and small molecules where activation of carboxylic acid groups on the ligand is not an issue.

1. Ensure that the protein or ligand is in an amine-free coupling buffer. 50–400 µg of protein or 1–5 nmoles of oligonucleotide in 100 µL coupling buffer is required for conjugation. Place on ice.
2. Resuspend the PureProteome™ Carboxy Magnetic Bead slurry by vortexing.
3. Pipet 100 µL of bead slurry into a 1.5 mL microcentrifuge tube.
4. Place the microcentrifuge tube into the PureProteome™ Magnetic Stand and allow the beads to migrate to the magnet. Remove the storage buffer with a pipette and discard.
5. Disengage the magnet and add 200 µL of activation/coupling buffer. Vortex vigorously for 20 seconds. Re-engage the magnet and allow the beads to migrate to the magnet. Remove the buffer with a pipette and discard.
6. Wash the beads two more times as in step 5.
7. Remove the microcentrifuge tube from the magnetic stand. Add 50-400 µg ligand in 80 µL activation/coupling buffer.
8. Incubate beads with continuous mixing for 30 minutes at room temperature.
9. Immediately before use prepare EDC (50 mg/mL) in activation/coupling buffer.
10. A pulse in a microcentrifuge may be required if liquid has collected in the microcentrifuge tube cap. Add 20 µL of the freshly prepared EDC solution to the beads. Vortex to mix well.
11. Incubate beads with continuous mixing for 0.5–4 hours at room temperature.
12. A pulse in a microcentrifuge may be required if liquid has collected in the microcentrifuge tube cap. Place the microcentrifuge tube into the magnetic stand and allow the beads to migrate to the magnet. Remove the supernatant with a pipette. This supernatant contains the unbound ligand fraction and can be saved for analysis if optimizing the protocol.
13. Disengage the magnet and wash the beads with 100 µL of activation/coupling buffer. Vortex to mix well, then re-engage the magnet and allow the beads to migrate to the magnet. Remove the supernatant with a pipette and discard. This wash can be saved if optimizing the protocol.
14. Disengage the magnet and add 500 µL of quench buffer (TBS, pH 8, 0.01% Triton® X-100 detergent) to the beads. Vortex vigorously for 20 seconds. Re-engage the magnet and allow the beads to migrate to the magnet. Remove the buffer with a pipette and discard.
15. Disengage the magnet and add 500 µL of quench buffer (TBS, pH 8, 0.01% Triton® X-100 detergent). Incubate for 30–60 minutes at room temperature. Re-engage the magnet and allow the beads to migrate to the magnet. Remove the buffer with a pipette and discard.
16. Disengage the magnet and add 500 µL of quench buffer (TBS, pH 8, 0.01% Triton® X-100 detergent). Vortex vigorously for 20 seconds. Re-engage the magnet and allow the beads to migrate to the magnet. Remove the buffer with a pipette and discard.
17. Wash the beads twice more as in step 16.
18. Remove the microcentrifuge tube from the magnetic stand. Add 100 µL storage buffer. Vortex to mix well and store the beads at 2–8 °C.

Specifications

PureProteome™ Carboxy Flexibind Magnetic Beads

	0.3 µm	1.0 µm	2.5 µm
Presentation	1% slurry (10 mg/mL) in 0.5% SDS and 0.05% sodium azide	1% slurry (10 mg/mL) in 0.5% SDS and 0.05% sodium azide	3% slurry (30 mg/mL) in 0.5% SDS and 0.05% sodium azide
Matrix/ particle form	Superparamagnetic material in a spherical polystyrene bead	Superparamagnetic material in a spherical polystyrene bead	Superparamagnetic material in a spherical polystyrene bead
Bead diameter	0.3 µm (nominal)	1.0 µm (nominal)	2.5 µm (nominal)
Storage	2–8 °C. Do not freeze.	2–8 °C. Do not freeze.	2–8 °C. Do not freeze.
Ligand Density	89–175 µmoles carboxylic acid/g beads	97–114 µmoles carboxylic acid/g beads	53–80 µmoles carboxylic acid/g beads
Shelf life	12 months from date of receipt	12 months from date of receipt	12 months from date of receipt

PureProteome™ Carboxy FlexiBind 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 and enter your catalogue number in the search box.

Product Ordering

Description	Qty/Pk	Catalogue No.
PureProteome™ Carboxy Flexibind Magnetic Beads		
0.3 µm beads	2 mL	LSKMAG03CBX02
	10 mL	LSKMAG03CBX10
1.0 µm beads	2 mL	LSKMAG1CBX02
	10 mL	LSKMAG1CBX10
2.5 µm beads	2 mL	LSKMAG25CBX02
	10 mL	LSKMAG25CBX10
PureProteome™ NHS FlexiBind Magnetic Beads Kit	1	LSKMAGN01
Contains 0.5 mL magnetic beads, equilibration, wash/coupling, and quench buffers, and Amicon® Ultra-0.5 devices.		
PureProteome™ NHS FlexiBind Magnetic Beads	4 × 0.5 mL	LSKMAGN04

Description	Qty/Pk	Catalogue No.
PureProteome™ Magnetic Stand, 8-well	1	LSKMAGS08
PureProteome™ Magnetic Stand, 15 mL	1	LSKMAGS15
PureProteome™ Streptavidin Magnetic Beads	2 × 1 mL	LSKMAGT02
	1 × 10 mL	LSKMAGT10
PureProteome™ Nickel Magnetic Beads	2 × 1 mL	LSKMAGH02
	1 × 10 mL	LSKMAGH10
PureProteome™ Protein A Magnetic Beads	2 × 1 mL	LSKMAGA02
	1 × 10 mL	LSKMAGA10
PureProteome™ Protein G Magnetic Beads	2 × 1 mL	LSKMAGG02
	1 × 10 mL	LSKMAGG10
PureProteome™ Albumin Magnetic Beads	1 × 10 mL	LSKMAGL10
PureProteome™ Albumin/IgG Depletion Kit	1	LSKMAGD12
Contains magnetic beads, buffer concentrate, and Amicon® Ultra-4 devices.		

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page on our web site at SigmaAldrich.com/TechService.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/Terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/Offices.

Document Template 00035533 Ver 1.0
20172057 Ver 3.0, Rev 21OCT2025, CJ