User Guide

PureProteome™ NHS FlexiBind Magnetic Bead Kit

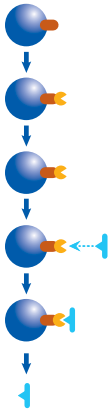
LSKMAGHDKIT

FOR RESEARCH USE ONLY

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

Introduction

PureProteome™ NHS Flexibind Magnetic Beads offer the flexibility to bind different ligands and allow permanent immobilization of proteins such as antibodies and other primary amine-containing biomolecules. Once customized by the user, these beads can be used for a variety of downstream applications such as affinity enrichment, protein purification, immunoprecipitation, and cell depletion.



PureProteome™ NHS Flexibind Magnetic Bead

Bind primary amine-containing reagents (e.g., proteins or antibodies)

Block residual active groups

Bind target protein

Wash

Elute target protein

Benefits

- Ready to use
- High density of NHS for coupling
- Form stable covalent bonds
- May be used over broad pH range (4.5–9.0) and temperature range (4–25 °C)

Components

PureProteome™ NHS FlexiBind Magnetic Bead Kit Catalogue No. LSKMAGN01

PureProteome™ NHS FlexiBind Magnetic Beads, 0.5 mL

Amicon® Ultra-0.5 30K Centrifugal Filter Device, 4 pk

Equilibration Buffer: 1 mM HCl, 5 mL

Wash/Coupling Buffer: Phosphate-buffered saline (PBS), pH 7.4, 25 mL

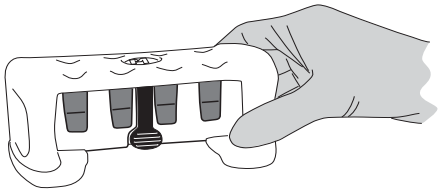
Quench Buffer: 100 mM Tris-HCl, 150 mM NaCl, pH 8.0, 5 mL

PureProteome™ NHS FlexiBind Magnetic Beads Catalogue No. LSKMAGN04

PureProteome™ NHS FlexiBind Magnetic Beads, 4 x 0.5 mL

Materials Required (but Not Supplied)

- 2 mL microcentrifuge tubes
- PureProteome™ Magnetic Stand (8-well)



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

Procedure for Using PureProteome™ NHS FlexiBind Magnetic Beads

The following protocol provides general guidelines for the coupling of proteins or antibodies to 100 µL of PureProteome™ NHS FlexiBind Magnetic Bead slurry. The protocol may be scaled up or down as desired. Optimization of the coupling conditions (protein concentration, coupling buffer, pH, and incubation time) for the ligand of interest is recommended.

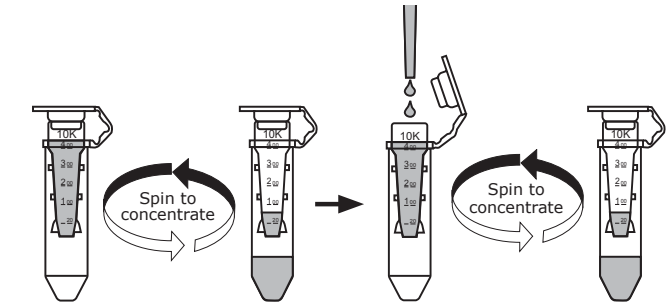
NOTES:

- Ensure that the magnetic beads are uniformly resuspended by vortexing or inverting. Mix the bead slurry between aliquots to ensure that the beads do not settle. The beads may appear cloudy when resuspended but this will not affect performance.
- For best performance, proteins should be at a concentration ≥ 2 mg/mL in an amine-free buffer. Buffers containing tris or glycine cannot be used. Refer to "Using Centrifugation for Concentration or Buffer Exchange" for instructions on using Amicon® Ultra-0.5 devices to exchange buffers.
- If using the PureProteome™ NHS FlexiBind Kit, PBS is provided and may be used as a wash/coupling buffer, but the user may need to optimize the wash/coupling buffer and pH in order to stabilize the ligand and maximize conjugation efficiency.
- Some alternative Coupling Buffers are listed below:
    - Potassium phosphate, 100 mM NaCl, pH 6.0
    - 150 mM Triethanolamine, pH 8.5
    - 200 mM NaHCO3, pH 8.3
    - 100 mM 2-(N-morpholino)ethanesulfonic acid (MES), pH 4.8
- An alternative Wash Buffer is 0.1 M Acetate buffer, 0.5 M NaCl, pH 4.5.
- An alternative Quench Buffer is 0.2 M Glycine-HCl, pH 2.5.
- The Equilibration Buffer (1 mM HCl) must be ice-cold.

Using Centrifugation for Concentration or Buffer Exchange (Optional)

If using the PureProteome™ NHS FlexiBind Kit, four Amicon® Ultra-0.5 30K devices and PBS are provided for buffer exchange of amine-containing buffers and/or concentration of proteins with molecular weights greater than 50,000–60,000. For smaller proteins, we recommend the Amicon® Ultra-0.5 10K device. A physical deadstop in the filter device prevents spinning to dryness and avoids potential sample loss. The concentrated/buffer-exchanged sample is collected by inverting the device into the second microcentrifuge tube and spinning again for a few minutes. The full user guide for the Amicon® Ultra-0.5 device can be found at SigmaAldrich.com. Enter Amicon Ultra-0.5 in the search box and search in the Technical Library.

1. Insert the Amicon® Ultra-0.5 device into one of the provided microcentrifuge tubes.
2. Add up to 500 µL of sample to the device and cap it.
3. Place capped device into the centrifuge rotor, aligning the cap strap toward the center of the rotor; counterbalance with a similar device.
4. Spin the device at 14,000 × *g* for approximately 5–10 minutes depending on the nominal molecular weight limit of the device used.
5. For applications that require buffer exchange, add 450 µL of the exchange buffer of choice and centrifuge again to concentrate the sample. Repeat this wash as required to ensure the desired exchange of buffer.



6. Remove the assembled device from the centrifuge and separate the Amicon® Ultra-0.5 device from the microcentrifuge tube.
7. To recover the concentrated sample, place the device upside down in a clean microcentrifuge tube. Place in centrifuge, aligning open cap towards the center of the rotor; counterbalance with a similar device. Spin for 2 minutes at 1,000 × *g* to transfer the concentrated sample from the device to the tube. For optimal recovery, perform the reverse spin immediately.

Alternatively, concentration and buffer exchange may be performed using a different method, such as protein precipitation.

General Coupling Protocol

1. Ensure that the protein or ligand is in an amine-free wash/coupling buffer and at a concentration ≥ 2 mg/mL. Place on ice. 100–200 µg of protein in 30–60 µL Wash/Coupling Buffer (PBS) is required for conjugation.
2. Resuspend the 20% NHS FlexiBind Magnetic Bead slurry by vortexing or inverting.
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. Immediately add 500 µL of ice-cold Equilibration Buffer (1 mM HCl), disengage the magnet, and 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. Immediately add 30-60 µL of ligand from step 1 and remove tube from the stand.
7. Incubate beads with continuous mixing for 1–2 hours at room temperature or overnight at 4 °C.
8. Place tube into magnetic stand and allow the beads to migrate to the magnet. Remove the unbound ligand and save for analysis if optimizing or calculating the bound ligand.
9. Add 500 µL of Quench Buffer (100 mM Tris-HCl, 150 mM NaCl, pH 8.0) disengage the magnet, and vortex vigorously for 30 seconds. Re-engage the magnet and allow the beads to migrate to the magnet. Remove the buffer with a pipette and discard.

10. Wash the beads an additional 4 times using 500 µL of Quench Buffer (100 mM Tris-HCl, 150 mM NaCl, pH 8.0) as described in step 9.
11. Add 500 µL of Quench Buffer (100 mM Tris-HCl, 150 mM NaCl, pH 8.0) and incubate for a minimum of 1 hour at room temperature. Place tube into magnetic stand and allow the beads to migrate to the magnet. Remove the buffer with a pipette and discard.
12. Resuspend the beads in 100 µL Wash/Coupling Buffer (PBS) or Tris-buffered saline (TBS) and store at 2–8 °C.

General Immunoprecipitation Protocol

The following protocol provides guidelines for immunoprecipitation and will require optimization.

1. Couple an antibody of choice to the PureProteome™ NHS FlexiBind Magnetic Beads as described in the General Coupling Protocol.
2. Prepare a cell lysate from a cell model appropriate for the target of interest.
3. In a microcentrifuge tube, dilute the cell lysate with Wash/Coupling Buffer (PBS) to approximately 1–2 mg/mL of total cell protein. A more concentrated cell lysate may be required for immunoprecipitation of low abundant targets.

   Optional: Users may choose to preclear the cell lysate at this step.
4. Remove 20-40 µL of the coupled magnetic beads prepared in step 1 and place in a microcentrifuge tube. The optimal amount of conjugated PureProteome™ NHS FlexiBind Magnetic Beads required for immunoprecipitation of the protein of interest should be empirically determined for each cell model and target.
5. Place the tube into the PureProteome™ Magnetic Stand and allow the beads to migrate to the magnet. Remove the buffer and discard.
6. Add 500 µL of Wash/Coupling Buffer (PBS) to the beads, disengage the magnet, and vortex for 20 seconds. Re-engage the magnet and allow the beads to migrate to the magnet. Remove the buffer with a pipette and discard.
7. Remove the tube from the rack and add 100–200 µL of cell lysate to the washed beads.
8. Incubate beads with continuous mixing for 2 hours at room temperature or overnight at 4 °C.
9. Place tube into magnetic stand and allow the beads to migrate to the magnet. Remove the supernatant (unbound fraction) and save for analysis if optimizing the protocol.
10. Add 500 µL of Wash/Coupling Buffer (PBS) to the beads, disengage the magnet, and vortex for 20 seconds. Re-engage the magnet and allow the beads to migrate to the magnet. Remove the buffer with a pipette and discard. Wash the beads 3–4 more times in this manner.

    Note: TBS or TBST (Tris-buffered saline with Tween® 20 surfactant) may be used for more stringent washing.
11. The captured immunocomplex can be analyzed by SDS-PAGE. Resuspend the beads in 15–45 µL of SDS-PAGE loading buffer and mix gently. This will allow for sufficient volume to run one to three lanes.
12. Heat the beads for 5 minutes at 95 °C. Place the tube into the PureProteome™ Magnetic Stand and allow the beads to migrate to the magnet. Remove the supernatant and save for analysis by SDS-PAGE.

Specifications

Presentation	20% (v/v) slurry in isopropanol
Matrix	N-hydroxysuccinimide (NHS) functional groups on silica-based beads
Particle form	Spherical
Bead diameter	10 µm (nominal)
Storage	2–8 °C, do not freeze
Ligand Density	>17 µmoles NHS per mL settled beads
Shelf life	Refer to expiration date on product label

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Troubleshooting/Optimization

Problem	Cause	Solution
No conjugation of protein to beads	Free amines (such as tris or glycine) in buffer	Perform a buffer exchange using the Amicon® Ultra-0.5 30K Centrifugal Filter Device provided in the kit (for proteins with molecular weights greater than 50,000–60,000. For smaller proteins, we recommend the Amicon® Ultra-0.5 10K device).
	Hydrolysis of the NHS amine-reactive groups on the magnetic beads	Use ice-cold equilibration and wash/coupling buffers and work quickly to equilibrate the beads and initiate coupling.
Poor conjugation of protein to beads	Optimization required	Vary the amount of ligand offered. Test alternative wash/coupling buffers.
Magnetic beads do not migrate to the magnet	Magnetic strength is not sufficient	The PureProteome™ Magnetic Stand is required for optimal performance.

Performance

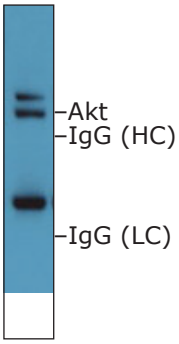

Figure 1: Immunoprecipitation with PureProteome™ NHS FlexiBind Magnetic Beads.

Anti-Akt/PKB (05-591) was coupled to the PureProteome™ NHS FlexiBind Magnetic Beads and used to immunoprecipitate Akt from an IGF-1 stimulated L6 cell lysate.

Product Ordering

Description	Qty/Pk	Cat. No.
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
PureProteome™ Magnetic Stand, 8-well	1	LSKMAGS08
PureProteome™ Magnetic Stand, 15 mL	1	LSKMAGS15
PureProteome™ Streptavidin Magnetic Beads	2 × 1 mL 1 × 10 mL	LSKMAGT02 LSKMAGT10
PureProteome™ Nickel Magnetic Beads	2 × 1 mL 1 × 10 mL	LSKMAGH02 LSKMAGH10
PureProteome™ Protein A Magnetic Beads	2 × 1 mL 1 × 10 mL	LSKMAGA02 LSKMAGA10
PureProteome™ Protein G Magnetic Beads	2 × 1 mL 1 × 10 mL	LSKMAGG02 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		
Amicon® Ultra-0.5 30K Centrifugal Device	8 24 96	UFC503008 UFC503024 UFC503096
Amicon® Ultra-0.5 10K Centrifugal Device	8 24 96	UFC501008 UFC501024 UFC501096

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.

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