MagnaLINK® 4FB Magnetic Beads

Cat. No.: M-1004

Storage: 2° – 8°C — Do Not Freeze.

DESCRIPTION

MagnaLINK 4FB Magnetic Beads are uniform, polymer encapsulated, super-paramagnetic particles activated with 4-formylbenzamide (4FB) on their surface. Based on **SoluLINK® bioconjugation technology**, these aromatic aldehydes react efficiently and rapidly with HyNic-modified biomolecules for high capacity immobilizations. The high surface area and low non-specific binding of MagnaLINK 4FB Magnetic Beads makes them ideal for covalent immobilization of HyNic-modified antibodies and other proteins, peptides, DNA, RNA, oligonucleotides, and other biomolecules. MagnaLINK 4FB Magnetic Beads benefit from a very uniform diameter (2.8 ± 0.2 microns) which makes them ideal for robotic applications and assays where batch-to-batch size uniformity and binding capacity are paramount. HyNic-peptide loading capacities routinely exceed 35 nmol/mg of beads, providing extremely high binding and fast magnetic response times. These qualities make MagnaLINK 4FB Magnetic Beads an affordable solution for automated, high throughput immobilization processes without the non-specific binding and other issues experienced with streptavidin-coated beads. MagnaLINK 4FB Magnetic Beads are supplied at 1% solids (10 mg/mL) in ultrapure water with 0.05% sodium azide.

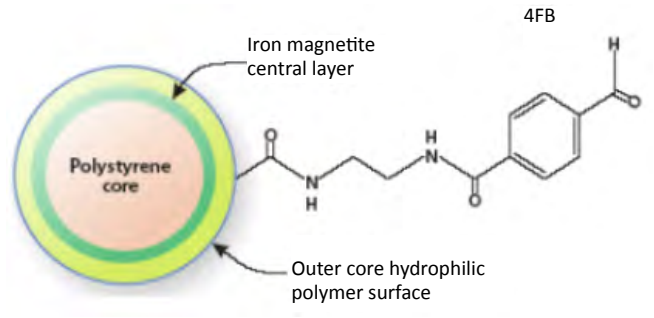

Figure 1. Cross-section of MagnaLINK 4FB Magnetic Beads.

PROTOCOL

Immobilization of HyNic-modified biomolecules to MagnaLINK 4FB Magnetic Beads

HyNic-modified biomolecules can be immobilized on MagnaLINK 4FB Magnetic Beads by simply mixing the desired amount of HyNic-modified biomolecule with the 4FB beads in conjugation buffer. For even faster and more efficient binding, 1/10th volume of TurboLINK™ Catalyst Buffer can be added to accelerate conjugate formation several-fold. Prior to immobilization of HyNic biomolecules, a wash step to equilibrate the 4FB beads in the appropriate conjugation buffer is recommended. Follow the general instructions below to wash the beads and equilibrate them in Conjugation Buffer prior to immobilization of the HyNic-modified biomolecule.

A. Washing procedure for MagnaLINK 4FB Magnetic Beads

Note: *The washing/equilibration procedure described here uses 1.0 mg of MagnaLINK 4FB Magnetic Beads. The amount of beads may be scaled up or down proportionally as desired.*

1. Resuspend MagnaLINK 4FB Magnetic Beads in their original vial using a vortex mixer to mix vigorously on the highest setting for 1 – 2 minutes. If a bath sonicator is available, the beads should also be sonicated for 1 – 2 minutes to ensure a monodisperse solution.
2. Transfer 1.0 mg (100 µL) of beads to a 1.5 mL microcentrifuge tube.
3. Place the tube on a magnet for approximately 2 minutes to pellet the beads, then carefully remove and discard the supernatant without disturbing the bead pellet using a P-200 pipet.
4. Remove the tube from the magnet and resuspend the beads in 250 µL of 1X Conjugation Buffer. Vigorous vortexing and/or sonication should be used to resuspend the bead pellet and ensure a monodisperse suspension. Addition of a non-ionic surfactant such as 0.05% Tween-20 is highly recommended at this step as it greatly improves the monodispersity of the suspension.
5. Place the tube on a magnet for approximately 2 minutes to pellet the beads, then carefully remove and discard the supernatant as before.
6. Repeat the wash step one additional time using 1X Conjugation Buffer + 0.05% Tween 20 and discard the supernatant.
7. Add 100 µL of 1X Conjugation Buffer (with or without 0.05% Tween-20, depending on the compatibility with the biomolecule to be conjugated) to the beads and vortex/sonicate vigorously to create a monodisperse suspension.

The beads are now ready for immobilization of HyNic-labeled biomolecules.

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B. Immobilization of HyNic-peptides

1. Add a sufficient volume of ultrapure water or 1X Conjugation Buffer to the HyNic-peptide to create a 10 mg/mL solution. Gently vortex until the peptide is completely dissolved.

   Note: *Some peptides, especially longer sequences containing a high proportion of hydrophobic amino acids, may require the addition of organic solvent for complete dissolution. DMF or DMSO may be added, if necessary, to assist in dissolution. These solvents will not adversely affect the bead performance and can be used at concentrations up to 75% for very hydrophobic peptides. Once the peptide is in solution, it is stable for approximately 1 week at 4 °C. For longer-term storage, freeze the peptide solution at -20 °C or lower for maximum stability.*

2. Transfer 1.0 mg (100 µL) of pre-washed MagnaLINK 4FB Magnetic Beads into a 1.5 mL microcentrifuge tube. A larger tube may be used for larger volumes of beads, such as a 15 mL Falcon conical tube, if desired.

3. Add approximately 20 µL (200 µg) of HyNic-peptide per 1 mg of beads. Alternatively, calculate the volume required for 35 nmol of peptide and add that amount to the beads.

4. Place the tube on a platform shaker and allow the immobilization reaction to proceed for 2 hours at room temperature with 1/10th volume of TurboLINK buffer, or overnight without TurboLINK.

   Note: *Ensure the agitation speed is sufficient to maintain the beads in solution during the conjugation reaction.*

5. Remove the reaction from the shaker and place on a magnetic stand for approximately 2 minutes.

6. Remove the supernatant carefully using a pipet without disturbing the pellet. The supernatant may be saved for later analysis if desired to confirm conjugation efficiency.

7. Wash the beads using 500 µL 1X Conjugation Buffer (+ 0.05% Tween-20 if possible). Vortexing and sonication should be used to ensure a homogenous solution and thorough washing.

8. Place the tube on the magnetic stand for approximately 2 minutes, then remove and discard the supernatant carefully without disturbing the pellet.

9. Repeat steps 7 and 8 one additional time, discarding the supernatant.

10. Resuspend the final conjugated bead pellet in 100 – 200 µL of a suitable buffer at a concentration of 5 – 10 mg/mL. The immobilized peptide on the solid phase is now ready for downstream applications. Buffers such as PBS, pH 7.20 or TBS, pH 7.4 are commonly used for bead resuspension.

    Note: *A bacteriostatic agent such as 0.05% sodium azide may be added to prevent microbial growth. Store the conjugated MagnaLINK Magnetic Beads refrigerated, but do not freeze the beads, as shearing will occur.*

Immobilization of antibodies to MagnaLINK 4FB Magnetic Beads

The process of immobilizing an antibody on MagnaLINK 4FB Magnetic Beads is similar to that of immobilizing a peptide. Antibodies should be prepared at a concentration of 2.5 mg/mL in a suitable carrier-free buffer, then modified with S-HyNic or Sulfo S-HyNic prior to immobilization. A minimum of 0.25 mg of antibody (100 µL) is suggested per 1.0 mg of MagnaLINK 4FB Magnetic Beads.

Note: *If the antibody is lyophilized, simply resuspend it in sufficient 1X Modification Buffer to obtain a 2.5 mg/mL solution. If the antibody is in solution at a concentration ≥ 2.5 mg/mL, transfer a volume equivalent to at least 0.25 mg to a 1.5 mL tube and dilute with 1X Modification Buffer to obtain a concentration of 2.5 mg/mL. If the antibody is in solution at a concentration < 2.5 mg/mL, it should be concentrated to 2.5 mg/mL using a VIVASPIN™ 500 diafiltration spin filter according to the manufacturer's instructions, or by following the Concentration of Dilute Antibody Solutions supplemental protocol.*

Protein concentration can be determined using any suitable protein determination assay such as a BCA or Bradford protein assay. Absorbance at 280 nm may also be used to determine antibody concentration prior to modification with S-HyNic, but not after modification. The incorporated HyNic groups absorb strongly at 280 nm and will skew the concentration value. After modification with S-HyNic, the BCA or Bradford assay must be used to determine protein concentration.

C. Buffer exchange of concentrated antibody

1. Prepare a 0.5 mL Zeba™ spin column by removing the bottom closure and loosening the red cap (do not remove the cap completely).

2. Place the column in a 1.5 mL microcentrifuge tube and centrifuge at 1,500 x g for 1 minute to remove storage solution.

   Important: *Ensure the centrifuge is set to "g" or RCF rather than RPM in all centrifugation steps.*

3. Using a lab marker, place a vertical mark on the side of the column where the compacted resin is slanted upward. Orient the column in the microfuge with this mark facing outward (away from the center of the rotor) in this and all subsequent centrifugation steps.

4. Slowly add 300 µL of 1X Modification Buffer to the top of the resin bed and loosely replace the cap.

5. Centrifuge at 1,500 x g for 1 minute and discard the flow-through from the collection tube.

6. Repeat steps 4 and 5 two additional times, discarding the flow through each time.

7. The column is now equilibrated with 1X Modification Buffer and ready for antibody loading.

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8. Place the spin column in a new 1.5 mL collection tube, remove the cap, and slowly apply 100 µL of antibody to the center of the compact resin bed. Avoid contact with the sides of the column when loading the sample, as the antibody solution must channel down through the resin for proper desalting.
9. Loosely cap the column and properly orient the tube in the centrifuge with the mark facing outward.
10. Centrifuge at 1,500 x g for 2 minutes.
11. Retain the desalted and buffer exchanged antibody solution in the bottom of the collection tube and proceed to modify the antibody with S-HyNic (section D).

    Note: *The antibody concentration should be confirmed at this step to ensure complete recovery of protein. Typical recoveries are around 90% using the Zeba desalting process.*

D. Modification of antibody with S-HyNic

1. Dissolve 5.0 mg S-HyNic in 1.0 mL anhydrous DMF. Alternatively, dissolve a 1.0 mg aliquot of S-HyNic in 200 µL of anhydrous DMF to create a 5.0 mg/mL solution.
2. Add 2.0 µL of S-HyNic/DMF solution (20 mole equivalents of HyNic over antibody) to 100 µL of desalted antibody solution at 2.5 mg/mL and mix well. Different amounts of antibody may be modified with the assistance of the Protein Modification Calculator, or by using 2.0 uL of S-HyNic/DMF solution per 0.25 mg of antibody.
3. Incubate the reaction at room temperature for 90 minutes.
4. Buffer exchange the HyNic-labeled antibody into 1X Conjugation Buffer using a new 0.5 mL Zeba spin column as described in section C.

   Note: *The antibody concentration may be checked at this step using a Bradford or BCA protein assay. Do not use the absorbance at 280 nm to determine concentration, as it will be inflated by the attached HyNic linkers.*

E. Antibody immobilization

1. Transfer 100 µL of washed MagnaLINK 4FB Magnetic Beads to a new 1.5 mL microcentrifuge tube.
2. Add 700 µL of 1X Conjugation Buffer (+ 0.05% Tween-20) to the 100 µL of beads.
3. Add 100 – 200 µL of HyNic-modified antibody (at ~2.5 mg/mL in 1X Conjugation Buffer) to the beads.
4. Add 1/10th volume of TurboLINK Buffer to the beads to catalyze the conjugation reaction.
5. Incubate the reaction on a platform shaker for 2 – 4 hours at room temperature.

   Note: *Ensure the agitation speed is sufficient to maintain the beads in solution during the conjugation reaction.*

After the conjugation step, proceed to washing the beads.

F. Washing procedure for MagnaLINK 4FB Magnetic Beads

1. Remove the reaction from the platform shaker and place the tube on a magnetic rack for approximately 2 minutes.
2. Carefully and slowly remove the supernatant with a pipet, leaving the beads undisturbed. The supernatant may be saved for later analysis of conjugation efficiency, if desired.
3. Add 1 mL of 1X PBS, pH 7.20 + 0.05% Tween-20 and vortex gently to wash the beads. Discard the supernatant.
4. Repeat steps 1-3 two additional times to remove any unbound antibody.
5. After discarding the final wash, resuspend the beads in 1.0 mL of 1X PBS. pH 7.20 with or without 0.05% Tween-20, or other suitable buffer.

The antibody coated beads are now at a final concentration of 1.0 mg/mL and may be used in downstream applications.