

DESCRIPTION

Bead- and column-based separation methods rely heavily on the speed and ease of affinity binding systems. Ligands such as streptavidin, antibodies and lectins are used both to capture specifically-tagged targets and for the isolation of cells and biomolecules that naturally express the ligand binding partner. The unique saccharide-binding properties of plant lectins, such as Concanavalin A (Con A) have made them useful for the labeling and isolation of glycan-presenting cells and glycoproteins in serum and cell lysate. Lectins have additionally been used in cell adhesion studies, to effect lymphocyte activation, and to explore carbohydrate-based therapeutics.

Our Con A-coated BioMag® Plus microparticles provide a convenient means for isolating mannosyl- and glucosyl-containing glycoproteins and polysaccharides from serum or cell lysate, or for investigating other lectin / glycan-mediated processes. BioMag® Concanavalin A has also been used for CUT&RUN, a chromatin profiling protocol that has several key advantages over ChIP. (see References)

CHARACTERISTICS

Concanavalin A (Con A) is covalently attached to functionalized BioMag® Plus particles for use in glycoprotein isolations from serum and cellular extracts.

Con A is a 104,000 Da protein comprised of four identical subunits, and exists as an active dimer or tetramer depending upon pH. Its carbohydrate binding partners are α -D-glucose and α -D-mannose with unmodified OH groups at C-3, C-4, and C-6, and terminal glucose residues of proteins and peptides. Con A agglutinates red blood cells (RBCs), interacts with immunoglobulin glycopeptides, and is a lymphocyte mitogen. It binds some bacteria.

Con A binding is mediated by metal ions, which stabilize its conformation. Each binding site requires calcium and manganese ions, and use of buffers with EDTA or other metal chelators will result in a loss of carbohydrate binding ability.

Mean diameter: ~1.0 μ m
Concentration: 5 mg/mL
Con A bound: Determined by A280

MATERIAL

Material Supplied

3mL or 10mL of Con A coated particles in 10mM PBS with 0.1% sodium azide

Material Required

1.5mL or 2mL microcentrifuge tubes

Mammalian serum: 0.4mL of a 1:20 dilution in PBS / test

Binding Buffer: 1x PBS + 0.1% NaN₃ + 1mM MgCl₂ + 1mM MnCl₂ + 1mM CaCl₂ (pH 7.4)

Wash Buffer: 1x PBS + 0.1% NaN₃ + 1mM MgCl₂ + 1mM MnCl₂ + 1mM CaCl₂ (pH 7.4) + 0.1% Tween® 20

Con A particle Elution Buffer: 5mM Tris (pH 8.0) + 0.15M NaCl + 0.05% SDS + 1M Glucose

Precision pipets with disposable tips to deliver 20-200 μ L, 200-1000 μ L

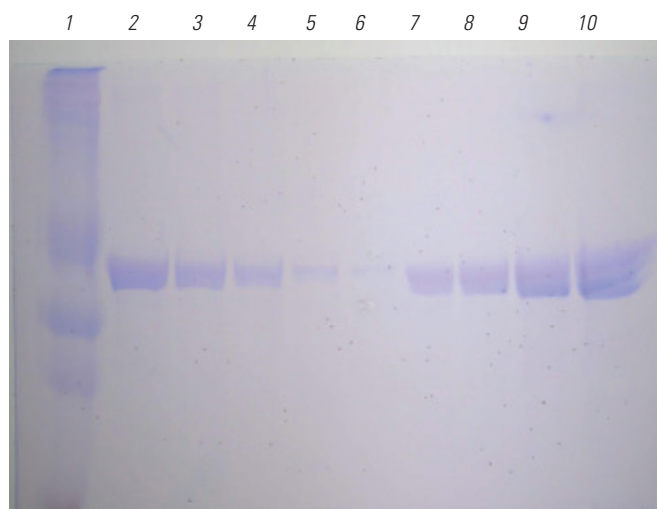
Microcentrifuge Tube Separator: 1.5mL Magnetic Separator (Catalog Code LS001) BioMag® Multi-6 Microcentrifuge Tube Separator (Catalog Code MS002)

Vortex mixer and tube rotator

PROCEDURE

Researchers are advised to optimize use of particles in any application.

1. Prepare 0.4mL of serum sample by diluting 1:20 with 10mM PBS.
2. Transfer 1mL of BioMag® Plus Con A particles to a clean microcentrifuge tube. Place the tube on a magnet to separate the particles from solution. Carefully remove and discard the solution.
3. Wash the particles by adding 1mL of Binding Buffer. Mix well.
4. Repeat the particle wash 1 more time. After the last wash, remove the supernatant.



Binding and elution of apo-transferrin using BioMag® Plus Con A magnetic particles. Sample elutes (lanes 2-6) from 1mL, 0.5mL, 0.25mL, and 0.125mL of BioMag® Plus Con A particles, respectively, are compared with aliquots of 0.3 mg/mL apo-transferrin stock (lanes 7-10) from 5 μ L, 10 μ L, 20 μ L, and 30 μ L.

5. Add 0.1mL of Binding Buffer to the serum sample from Step 1. Add the sample to the particles and mix well by inversion or vortex to resuspend the particles.
6. Place the sample on a tube rotator and mix for 10-30 minutes at room temperature.
7. Remove the sample from the rotator and place in a magnetic separator. Carefully remove the cleared supernatant.
8. Wash the particles by adding 0.5mL of Wash Buffer. Mix well by inversion or by vortex mixing.
9. Repeat Steps 7-8. Resuspend the particles with 0.5mL of Wash Buffer and place on tube rotator for 5 minutes.
10. Repeat Steps 7-9.
11. Replace the tube of particles on the magnetic separator and carefully remove / discard the supernatant.
12. Add 250µL of Elution Buffer to the particles. Mix the tube to resuspend the particles and place the tube on rotator for 10-30 minutes at room temperature.
13. Replace the tube of particles on the magnetic separator and carefully remove the eluate and transfer to a clean microcentrifuge tube for later use or storage.
14. Repeat Steps 12-13. Eluates may be pooled and precipitated. Store eluates on ice for immediate use or freeze for long-term storage.

NOTES

- Avoid the use of reagents with EDTA or other metal chelators, as this will reduce the effectiveness of the Binding Buffer.
- Protease Inhibitors may be used when sensitive glycoproteins are isolated.
- Low glycoprotein recovery may be attached by either increasing the elution incubation time beyond 10 minutes, and / or by boiling particles in 200µL of SDS-PAGE sample buffer for 5 minutes and then magnetically separating the particles from the eluate. (Note: Boiling may detach some lectins and may also release nonspecifically bound proteins.)
- Run eluate samples on an SDS-PAGE 4-20% Tris-Glycine electrophoresis gel and stain the glycoprotein bands using the GlycoGel Stain Kit (Polysciences' Catalog Code 24693) to visualize.
- After GlycoGel staining, stain the gel using Coomassie G250 (1mL or 2mL of 0.5% Coomassie G250 in 50% methanol and 10% acetic acid) to visualize other protein bands.
- The removal of albumin and IgG from serum samples may improve the isolation of low concentration glycoproteins. If desired, use the BioMag® ProMax Albumin Removal Kit (Catalog Code BP658) and / or the BioMag® ProMax Serum IgG Removal Kit (Catalog Code BP659).

REFERENCES

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3. Sparbier, K., T. Wenzel, M. Kostrzewa. 2006. *Exploring the binding profiles of Con A, boronic acid and WGA by MALDI-TOF / TOF-MS and magnetic particles*. J Chrom B Analyt Technol Biomed Life Sci, 840(1):29-36.
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TRADEMARKS

BioMag® is a registered trademark of Polysciences, Inc.

Tween® is a registered trademark of ICI Americas, Inc.

STORAGE AND STABILITY

Store at 2-8°C. Freezing, drying, or centrifuging particles may result in irreversible aggregation and loss of binding activity.

SAFETY

This particle suspension contains sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Upon disposal of material, flush with a large volume of water to prevent azide accumulation. Please consult the Safety Data Sheet for more information.

This product is for research use only and is not intended for use in humans or for *in vitro* diagnostic use.

ORDERING INFORMATION

Cat. Number	Description	Sizes
BP531	BioMag®Plus Concanavalin A	3mL or 10mL

RELATED PRODUCTS

Cat. Number	Description	Size
LS001	1.5mL Magnetic Separator	1 each
MS002	BioMag® Multi-6 Microcentrifuge Tube Separator	1 each
BP658	BioMag® ProMax Albumin Removal Kit	1 kit
BP659	BioMag® ProMax Serum IgG Removal Kit	1 kit

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