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BioMag®Plus Concanavalin A

Catalog Number: 86057

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. The BioMag®Plus magnetic particle format provides high surface area, and permits easy and efficient separations.

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 is 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:	~1.0µm
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% NaN_3 + 1mM $MgCl_2$ + 1mM $MnCl_2$ + 1mM $CaCl_2$ (pH 7.4)
- Wash Buffer: 1x PBS + 0.1% NaN_3 + 1mM $MgCl_2$ + 1mM $MnCl_2$ + 1mM $CaCl_2$ (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 20-200µl, 200-1000µl
- Microcentrifuge Tube Separator:
  BioMag® Solo-Sep Microcentrifuge Tube Separator
  (Cat. # 8MB4112S)
  BioMag® Multi-6 Microcentrifuge Tube Separator
  (Cat. # 8MB4111S)
  BioMag® Multi-32 Microcentrifuge Tube Separator
  (Cat. # 84106S)
- 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.
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.

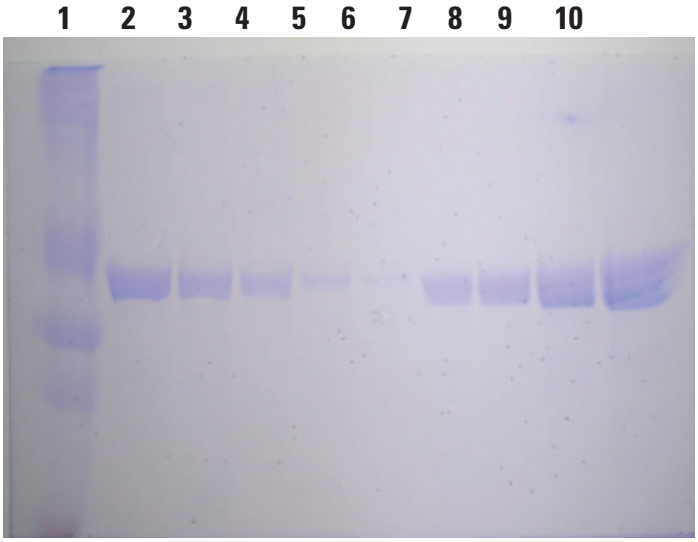

Figure 1: *Binding and elution of apo-transferrin using BioMag®Plus Con A magnetic particles. Sample elutes (lanes 2-6) from 1ml, 0.5ml, 0.25ml, 0.125ml and 0.05ml of BioMag®Plus Con A particles, respectively, are compared with aliquots of 0.3 mg/ml apo-transferrin stock (lanes 7-10) from 5µl, 10µl, 20µl and 30µl.*

NOTES

1. Avoid the use of reagents with EDTA or other metal chelators, as this will reduce the effectiveness of the Binding Buffer.
2. Protease Inhibitors may be used when sensitive glycoproteins are isolated.
3. Low glycoprotein recovery may be increased 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.)
4. Run eluate samples on an SDS-PAGE 4-20% Tris-Glycine electrophoresis gel and stain the glycoprotein bands using the GlycoGel Stain Kit (Cat. # 24693) to visualize.
5. 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.
6. The removal of albumin and IgG from serum samples may improve the isolation of low concentration glycoproteins. If desired, use the ProMax Albumin Removal Kit (Cat. # 24351) and / or the ProMax Serum IgG Removal Kit (Cat. # 24352).

ORDERING INFORMATION

Cat. #	Description	Sizes
86057-3	BioMag®Plus Concanavalin A	3ml
86057-10	BioMag®Plus Concanavalin A	10ml

REFERENCES

1. **Lotan, R., G.L. Nicholson.** 1979. Purification of cell membrane glycoproteins by lectin affinity chromatography. *Biochem Biophys Acta*, 559(4):329-376.
2. **Payne, M.J., S. Campbell, R.A. Patchett, R.G. Kroll.** 1992. The use of immobilized lectins in the separation of *Staphylococcus aureus, Escherichia coli, Listeria* and *Salmonella spp.* from pure cultures and foods. *J Appl Bacteriol*, 73(1):41-52.
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 Chromatogr B Analyt Technol Biomed Life Sci*, 840(1):29-36.
4. **Yahara, I., G.M. Edelman.** 1972. Restriction of the mobility of lymphocyte immunoglobulin receptors by Concanavalin A. *PNAS*, 69(3): 608-612.
5. **Zem, G.C., et al.** 2006. Microbead analysis of cell binding to immobilized lectin: an alternative to microarrays in the development of carbohydrate drugs and diagnostic tests. *Acta Histochem*, 108(4): 311-317.

STORAGE AND SAFETY

Storage Store at 4˚C. Freezing, drying, or centrifuging BioMag® may result in irreversible aggregation and loss of binding activity. Washing BioMag® SelectaPure anti-Human CD11b particles in sterile media to remove preservative prior to use is recommended. Using a magnetic separation unit for washing instead of centrifugation is also strongly recommended.

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.

These products are for research use only and are not intended for use in humans or for *in vitro* diagnostic use.

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