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TECHNICAL DATA SHEET 759

BioMag®Plus Wheat Germ Agglutinin

Catalog Number: 86054

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 wheat germ agglutinin (WGA) 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 WGA-coated BioMag®Plus microparticles provide a convenient means for isolating N-acetylglucosamine-containing glycoproteins 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

Wheat germ *(Triticum vulgaris)* agglutinin is covalently attached to functionalized BioMag®Plus particles for use in glycoprotein isolations from mammalian serum and cellular extracts.

WGA is a 36,000 Da protein comprised of two identical subunits. Its primary sugar-binding partner is N-acetylglucosamine (GlcNAc), and it binds most securely to GlcNAc dimers and trimers. WGA is a binder of gram-positive bacteria, via GlcNAc moieties in the peptidoglycan layer of the cell wall. WGA also interacts with saccharides with a terminal GlcNAc, chitobiose, or sialic acid residues. WGA does not contain protein-bound carbohydrate, and is not blood group specific.

Mean Diameter:	~1.5µm
Concentration:	5mg/ml
WGA bound:	Determined via A280

MATERIAL

Material Supplied

- 3ml or 10ml of WGA coated particles in 10mM PBS with 0.1% BSA + 0.075% NaN_3 + 0.004% EDTA

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 + 1 mM $MgCl_2$ + 1 mM $MnCl_2$ + 1 mM $CaCl_2$ (pH 7.4)
- Wash Buffer - 1x PBS + 0.1% NaN_3 + 1 mM $MgCl_2$ + 1 mM $MnCl_2$ + 1 mM $CaCl_2$ (pH 7.4) + 0.1% Tween® 20
- WGA particle Elution Buffer - 10mM Tris (pH 8.0) + 0.15 M NaCl + 0.1% SDS + 0.2 M N-acetyl glucosamine
- Precision pipets with disposable tips to deliver 20-200 µl, 200-1000 µl
- Microcentrifuge Tube Separator:
  BioMag® Solo-Sep Microcentrifuge Tube Separator, single sample (Cat. #8MB4112S)
  BioMag® Multi-6 Microcentrifuge Tube Separator, six samples (Cat. # 8MB4111S)
  BioMag® Multi-32 Microcentrigue Tube Separator, thirty-two samples (Cat. #84106S)
- Vortex mixer and tube rotator

PROCEDURE

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

1. Prepare 0.4mL of sample (e.g. serum) by diluting 1:20 with 10mM PBS.
2. Transfer 1mL of BioMag®Plus WGA 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 particles by adding 1mL of Binding Buffer and mix well.
4. Repeat the particle wash. 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 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 0.5 mL of Wash Buffer. Mix well by inversion or by vortex mixing.
9. Repeat Steps 7-8. Resuspend the particles with 0.5 mL 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 250 µL of Elution Buffer to the particles. Mix the tube to resuspend the particles and place the tube on rotator for 10 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. Store eluates on ice for immediate use or freeze for long-term storage.

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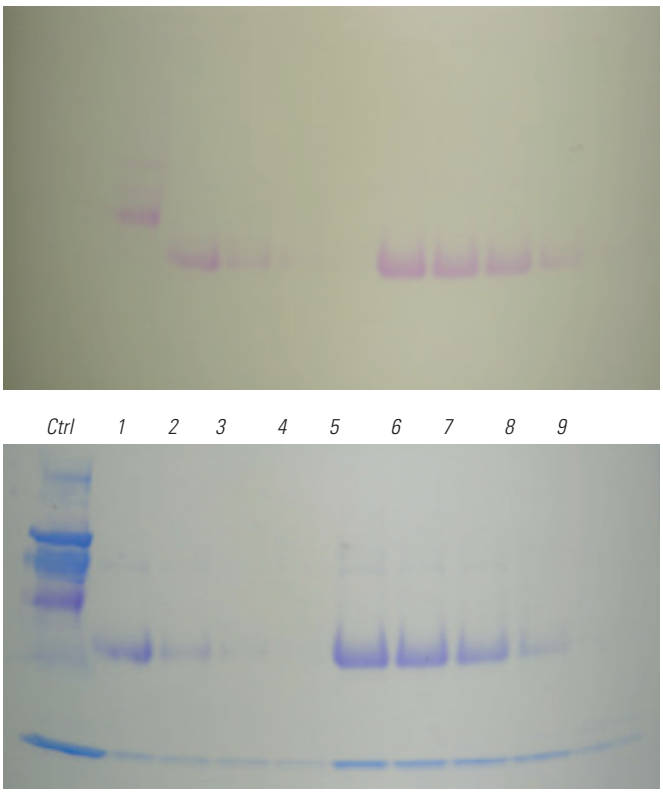

Figure 1: 4-20% Tris Glycine SDS-PAGE gel electrophoresis gels displaying staining of eluted tryspin inhibitor (Lanes 5, 6, 7, 8, and 9) using different volumes of BioMag®Plus WGA particles (1ml, 0.75ml, 0.5ml, 0.25ml, and 0.1ml). GlycoGel Glycoprotein staining (left) and restraining with Coomassie G250 (right). Lanes numbered 1, 2, 3, and 4 are titrated trypsin inhibitor control samples.

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 (Polysciences' 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 BioMag® ProMax Albumin Removal Kit (Cat. #24351) and / or the BioMag® ProMax Serum IgG Removal Kit (Cat. #24352).

ORDERING INFORMATION

Cat. #	Description	Sizes
86054-3	BioMag®Plus Wheat Germ Agglutinin	3ml
86054-10	BioMag®Plus Wheat Germ Agglutinin	10ml

REFERENCES

1. **Larsen, K., M.B. Thygesen, F. Guillaumie, W.G. Willats, K.J. Jensen.** 2006. Solid-phase chemical tools for glycobiology. *Carbohydr Res,* 341(10):1209-1234.
2. **Lotan, R., G.L. Nicholson.** 1979. Purification of cell membrane glycoproteins by lectin affinity chromatography. *Biochem Biophys Acta,* 559(4):329-376.
3. **Molday, R.S., S.P. Yen, A. Rembaum.** 1977. Applications of magnetic microspheres in labeling and separation of cells. *Nature*, 268(5619):437-438.
4. **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.

STORAGE AND SAFETY

Storage Store at 4˚C. Freezing, drying, or centrifuging BioMag® 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.

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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