Concanavalin A Magnetic Beads

Product 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 pres enting 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. Concanavalin A magnetic beads has also been used for CUT&RUN, a chromatin profiling protocol that has several key advantages over ChIP.

Product Features

Composition	Concanavalin A
Magnetization	Superparamagnetic
Particle size	-1um
Concentration	10 mg/mL
Application	Isolating glycoproteins，CUT&RUN,
Storage Condition	Store at 4°C for 2 years.

Protocol

Researchers are advised to optimize use of beads in any application

Material Required

1.5mL or 2mL microcentrifuge tubes

Mammalian cells(10000-100000 cells)

Binding Buffer: 1x PBS + 1mM $MgCl_2$ + 1mM $MnCl_2$ + 1mM $CaCl_2$ (pH7.4)

Wash Buffer: 1x PBS + 1mM $MgCl_2$ + 1mM $MnCl_2$ + 1mM $CaCl_2$ (pH 7.4) + 0.1% Tween® 20

Elution Buffer; 5mM Tris (pH 8.0) + 0.15M NaCl + 0.05% SDS + 1M Glucose

Preparation of Cells

1.Prepare mammalian cells （10000-100000 cells）sample，Centrifuge at 600 x g for 3-5 minute at room temperature., Carefully remove the supernatant

2.Add 90ul of Binding Buffer to cells, Mix the tube to resuspend the cells. Centrifuge at 600 x g for 3-5 minute at room temperature., Carefully remove the supernatant

3. Add 90μL Binding Buffer to the cells and resuspend Carefully.

Preparation of Magnetic Beads

4.Transfer 10uL of Con A Magnetic Beads to a clean microcentrifuge tube. Place the tube on a magnet to separate the beads from solution. Carefully remove and discard the solution.

5. Wash the beads by adding 200μL of Binding Buffer. Mix well.

6. Repeat the particle wash 1 more time. After the last wash, remove the supernatant.

7. Add the cells sample from Step 3 to the beads and mix well by inversion to resuspend the beads.

8. Place the sample on a tube rotator and mix for 10-30 minutes at room temperature.

9. Remove the sample from the rotator and place in a magnetic separator. Carefully remove the cleared supernatant.

10. Wash the beads by adding 0.5mL of Wash Buffer. Mix well by inversion or by vortex mixing.

11. Repeat Steps 9-10. Resuspend the beads with 0.5mL of Wash Buffer and place on tube rotator for 5 minutes.

12. Repeat Steps 9-10.

13. Replace the tube of beads on the magnetic separator and carefully remove / discard the supernatant.

14. Add 50-250µL of Elution Buffer to the beads. Mix the tube to resuspend the beads and place the tube on rotator for 10-30 minutes at room temperature.

15. Replace the tube of beads on the magnetic separator and carefully remove the eluate and transfer to a clean microcentrifuge tube for later use or storage.

16. Repeat Steps 14-15. 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 beads in 200µL of SDS-PAGE sample buffer for 5 minutes and then magnetically separating the beads from the eluate. (Note: Boiling may detach some lectins and may also release nonspecifically bound proteins.