Concanavalin A Magnetic Beads

Product description

Concanavalin A (ConA) is a plant mannose/glucose-binding lectin isolated from the seeds of cereal plants such as giant bean (Jack bean, Canavalia ensiformis), rice, bean, wheat, etc., and its monomer can bind one Ca^{2+} and one Mn^{2+} which Contains a glycosyl-binding site with high affinity for terminal α-D-mannosyl and α-D-glucosyl residues.

Concanavalin A Magnetic Beads (Canoladin A magnetic beads) is covalently coupled with canadolin A and superparamagnetic nanoscale magnetic beads, which can quickly, efficiently, sensitively and specifically bind to glycoproteins, glycolipids, polysaccharides and other glycosylation modification molecules, mainly used to isolate cells or for isolation of cell or tissue lysate or glycoprotein in serum glycosylation modification molecules and other experiments, as well as nuclease-targeted cleavage and release of chromatin (Cleavage Under Targets & Release Using Nuclease, CUT&RUN)。

Characteristics	Description
Product content	10 mg/mL magnetic beads in specific protective buffer
Beads size	~1 μm
Magnetization	Superparamagnetic
Coupled protein	Concanavalin A
M.W. of protein	~102 kDa (tetramer)
Specificity	Glycan and glycoconjugates
Application	Isolating cells or glycoproteins, CUT&RUN

Table The main related indicators of Concanavalin A Magnetic Beads.

Components and storage conditions

Components	K1308-1 mL	K1308-5 mL
Concanavalin A Magnetic Beads	1 mL	5 mL
Store the components dry at 4 °C for 12-18 months.		

Experimental manipulation

The following operations take purification of glycoproteins or isolation of cells as an example, and the

CUT&RUN experiment needs to prepare cells and subsequent experimental items according to the corresponding experimental methods.

1. Preparation of buffers (bring your own)

Buffer	Components
Binding Buffer	20 mM Hepes (pH 7.4), 150 mM NaCl (optional), 1 mM $MgCl_2$, 1 mM $MnCl_2$, 1 mM $CaCl_2$
Wash Buffer	20 mM Hepes (pH 7.4), 150 mM NaCl (optional), 1 mM $MgCl_2$, 1 mM $MnCl_2$, 1 mM $CaCl_2$, 0.1% Tween-20
Elution Buffer	5 mM Tris (pH 8.0), 150 mM NaCl, 1 M Glucose

Notes:

1: The use of PBS buffer may produce precipitation; it is recommended to use Hepes buffer. Before use, equilibrate all solutions to room temperature. The 150 mM NaCl in both Binding Buffer and Wash Buffer can be added selectively, and 150 mM NaCl is not recommended for experiments that have little relevance to cell viability, such as CUT&RUN, or 150 for subsequent cell culture or cell function studies mM NaCl. The binding and precipitation efficiency of cells is higher when 150 mM NaCl is not added, and the osmotic pressure of cells can be better maintained when 150 mM NaCl is added, so that the cells are in a better state.

2: Elution Buffer needs to be optimized according to the type of glycoprotein or the degree to which the glycoprotein binds to the ConA beads.

3: Since the cells are very tightly bound to the ConA beads, it is not recommended to use this eluate for elution of isolated cells.

2. Sample handling.

   a) Prepare mammalian cells (1.0×10^{4}~1.0×10^{5}pcs), centrifuge (4°C, 600×g, 3~5 min) and carefully aspirate the supernatant.

   b) Add 90 μL of Binding Buffer, mix well and resuspend the cells, centrifuge and collect (4°C, 600×g, 3~5 min), carefully aspirate the supernatant.

   c) Add 90 μL of Elution Buffer and mix well to resuspend the cells. (Optional simultaneous addition of protease inhibitor (K1007) to inhibit protein degradation)

3. ConA magnetic bead pretreatment

   a) Gently pipette the ConA beads to fully suspension, place 10 μL of the bead suspension in a new 1.5 mL centrifuge tube and stand on the magnetic stand for 1 min and discard the supernatant after the magnetic beads adsorb to the side wall of the centrifuge tube.

   b) Add 200 μL of Binding Buffer, gently pipette the resuspended magnetic beads, then stand on the magnetic stand for 1 min, and discard the supernatant after the magnetic beads adsorb to the side

wall of the centrifuge tube.

c) Repeat the previous step (3-b) once.

Note 1: When multiple samples, take the total amount of magnetic beads, combine the washing treatment, and then divide it into each sample.

Note 2: Activated magnetic beads should be used daily.

4. Sample binding

Add the prepared cell sample to the pretreated beads, gently pipette the resuspended beads, then incubate on an inverted mixer (room temperature 30 min), then stand on a magnetic stand for 1 min, wait for the magnetic beads to adsorb to the side wall of the centrifuge tube, carefully discard the supernatant, and the remaining protein bead complex in the centrifuge tube is the protein-magnetic bead complex.

Note 1: During the incubation process, if ConA magnetic beads clump or flaky, it is normal to do so, which will not affect the experimental results.

Note 2: To ensure that the sample is fully bound to the beads, increase the amount of ConA beads and extend the incubation time.

5. Elution of glycoproteins.

a) Add 500 μL of Wash Buffer to the protein-bead complex and gently pipette the beads to allow them to be fully suspended, then stand on a magnetic stand for 1 min and remove the supernatant after the beads adsorb to the side wall of the centrifuge tube. Repeat this step two more times.

b) Add 50~250 μL of Elution Buffer to the protein-magnetic bead complex in the upward step, incubate on a flip mixer (room temperature 10~30 min), then stand on a magnetic stand for 1 min, and collect the supernatant after the magnetic beads are adsorbed to the side wall of the centrifuge tube, which is the protein of interest.

Notes

1. Before the use of this product, it should be properly and fully resuspended, that is, inverted several times to make the magnetic beads mix evenly, the mixing operation should be gentle, not violently vortex and shake, etc., to avoid ConA denaturation, etc.

2. When used for precipitation or purification, it is recommended to set up appropriate positive and negative control groups.

3. The type and size of the molecules to be bound will affect the binding efficiency, it is recommended to determine the number of magnetic beads for each specific application by dilution and consider

increasing the amount of magnetic beads to 2-3 moles of the molecules to be bound to ensure sufficient binding.

4. ConA requires the presence of Ca^{2+} and Mn^{2+} ions to be active, so reagents containing EDTA, or other metal ion chelators should be avoided during the experiment.

5. Glycoprotein samples should be purified as soon as possible after collection and should always be placed at 4°C or in an ice bath to slow glycoprotein degradation. To effectively inhibit protein degradation, an appropriate amount of protease inhibitor cocktail can be added to the protein sample.

6. If an instrument such as a vacuum pump is used to aspirate the supernatant, attention should be paid to the suction strength of the vacuum pump to avoid excessive suction and suction of the magnetic beads.

7. When incubated with cells, ConA beads may aggregate, which is normal and does not affect the normal use of magnetic beads.

8. This product contains 0.05% NaN_3 in the preservation solution.

9. This product is for scientific purposes only.

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