Protein immobilization using PureCube Streptavidin MagBeads

Binding of biotinylated soluble or membrane bait proteins to magnetic beads for pull-down assays

1. Description

PureCube Streptavidin MagBeads are ferrimagnetic spheres covered with 6% agarose and coupled with streptavidin allowing efficient immobilization of biotinylated proteins. Magnetic particles are used to easily separate the beads from the surrounding liquid with the help of a magnet, making PureCube Streptavidin MagBeads perfectly suitable for batch applications. Due to the agarose surface and the high specificity of streptavidin, the beads show a very low non-specific protein binding.

This protocol describes the application of PureCube Streptavidin MagBeads for binding pre-purified biotinylated membrane proteins, preferably stabilized within nanodiscs consisting of biotinylated copolymers, and biotinylated soluble proteins.

2. Required material and recommendations

The table below lists all recommended buffers for protein enrichment with PureCube Streptavidin MagBeads (2-0209-001 or 2-0209-005). Please note that buffers that are provided as 10-fold concentrated solutions (10x) have to be diluted to obtain single concentrated buffers. To achieve this, mix one part of 10-fold concentrated buffer with nine parts of deionized water.

Recommended buffers/solutions		Cat. No.	Volume
Equilibration/Wash buffer	NativeMP™ Wash Buffer (10x) 200 mM HEPES pH 7.5, 1.5 M NaCl	91001	100 mL

For fast and simple separation of magnetic beads from buffer or sample, we recommend the application of our Cube MagBead Separator (Cat. No. 16941) if the pull-down occurs in reaction tubes (1.5-2 mL). With the Magnetic Separator several samples can be processed in parallel. If a larger number of samples should be purified, we recommend the use of round-bottom 96-well plates and an appropriate magnetic device.

Binding capacity of different biotinylated proteins per mL pure beads*	
Membrane protein stabilized with a biotinylated copolymer nanodisc (45 kDa)	3 mg/mL
Avi-tagged protein (~50 kDa)	≥ 50 mg/mL
Biotinylated BSA	≥ 20 mg/mL

*Note that binding is protein dependent.

You can adjust the volume of magnetic beads according to your target protein concentration. For proteins >150 kDa, described binding capacities might not be reached under the conditions described. Optimization might be necessary in this case (e.g. an increase of bead volume).

3. Protocol

3.1. Binding of biotinylated proteins

- This protocol is optimized for working at room temperature.
- 20 µL of the 25 % suspension contain 5 µL magnetic beads.
- Free biotin can strongly decrease binding capacity. Make sure to remove excess biotin using gel filtration or dialysis if possible.

3.1.1. Resuspend the magnetic beads by pipetting up and down. For bead loading e.g. with a **250 µL membrane protein sample (e.g. 170 µg/ml)**, pipette **60 µL of the 25 % suspension** (15 µL beads) into a reaction tube, place it on the magnetic separator to separate the beads and remove the supernatant.

3.1.2. Equilibrate beads in **500 µL equilibration buffer**. Separate the beads in the magnetic separator and remove the supernatant. Repeat this step twice.

3.1.3. Resuspend the magnetic beads with the **250 µL sample** containing the target protein. Keep the beads in suspension by gently inverting them using an end-over-end shaker or placing the tube in a thermoshaker at 1000-1300 rpm. Incubate for **30 min**.

Note that the binding efficiency can vary depending on the number of biotins attached to the protein of interest and the protein itself. If insufficient binding occurs, increase the incubation time up to 120 min and/or increase the amount of MagBeads used.

3.1.4. Place the reaction tube in the magnetic separator and carefully remove the supernatant.

3.1.5. Remove tube from the magnet and add **500 µL wash buffer**. Resuspend carefully and place the reaction tube in the magnetic separator to collect the beads. Remove the supernatant and repeat this step twice.

3.1.6 Store the loaded beads in buffer until further use for pull-down assays or immunoprecipitation.

3.2. Pull-down of proteins

3.2.1. Mix **300 µL sample** containing your binder/antigen with **5 µL** of prepared MagBeads (3.1.) and incubate for **1 h**.

3.2.2. Place the reaction tube in the magnetic separator and carefully remove the supernatant.

3.2.3. Remove tube from the magnet and add **500 µL wash buffer**. Resuspend carefully and place the reaction tube in the magnetic separator to collect the beads. Remove the supernatant and repeat this step twice.

Note that for membrane proteins stabilized in copolymer nanodiscs, more washing steps might be necessary to effectively wash off unspecifically bound proteins.

3.2.4. Elution can differ depending on the bait protein. For pull-down experiments with subsequent SDS-PAGE and/or Western blot elution can be performed with SDS sample buffer (a) and for immunoprecipitation (pull-down using antibodies) elution can either be performed according to (a) or using an acidic buffer (b).

a) Elution with SDS sample buffer

- ***Soluble proteins***: Apply SDS sample buffer and heat the sample at 95-100 °C for 10 min. Immobilized Streptavidin will denature under these conditions and detach from the magnetic beads, leading to an additional band at ~13 kDa during SDS-PAGE analysis.

- ***Membrane proteins***: Apply SDS sample buffer and heat the sample at 46 °C for 45 min. It is possible that immobilized Streptavidin might denature and an additional band at ~13 kDa can appear in your SDS-PAGE analysis.

b) Elution after immunoprecipitation

- To elute bound antigen, add 50 µl of a low pH buffer, e.g. 100 mM glycine-HCl (pH 2.0), to your MagBeads and incubate the sample with mixing for 5-10 min. Place the reaction tube in the magnetic separator to collect the beads and carefully transfer the supernatant to a fresh reaction tube.

4. Troubleshooting

No or weak binding of bait protein to PureCube Streptavidin MagBeads	
Free biotin or contamination with other biotinylated proteins.	Remove excess biotin or protein contaminants from your purified biotinylated bait protein via dialysis, filtration, or size exclusion chromatography before loading the beads with bait protein.
Insufficient biotinylation efficiency.	Verify biotinylation e.g. via a dot blot or Western blot.
Degradation of biotinylated protein	Use bait protein as fresh as possible. If stored at -20 °C or -80 °C, avoid repeated freeze-thaw cycles.
Contaminating proteins & poor recovery	
Recovered protein is degraded.	Add protease inhibitors to prevent proteolysis of prey protein
Nonspecific bands in eluted sample.	Increase number of wash cycles. Try increasing ionic strength of your washing solution or other additives to reduce nonspecific binding. Add DNase to reduce lysate viscosity.
Protein not properly bound to binding partner	Check if co-factors (e.g. ATP or metal ions) are necessary for an interaction between binding partners and add them to your mixture.