Big Scale Purification of Affinity-tagged Proteins using PureCube (GST, NTA, IDA, INDIGO), HiCap (Strep-Tactin®), and HighSpec (Rho) Affinity MagBeads

Overview

Handling sample sizes of up to 5 liters presents challenges with classic affinity agarose/ cartridge methods, which struggle with scalability. In contrast, magnetic beads (MagBeads) offer a streamlined alternative, overcoming the limitations of classic agarose-based purification and providing a more efficient and versatile solution for large-scale molecular studies. Our custom magnets play a pivotal role in efficiently and effectively retaining a maximum of recombinant protein.

We recommend using the GentleLys - Native Cell Lysis Buffers to extract your protein of interest. Both GentleLys - Stabilize and GentleLys - Dissolve are non-denaturing buffers, but Stabilize has the added advantage of allowing for an extraction and stabilization of your protein in its native conformation.

You can find them here:
https://cube-biotech.com/products/proteomics/cell-lysis-buffers/gentlelys-native-cell-lysis-buffer/18803

Note

GentleLys Buffers are only suitable for eukaryotic cells and plant protoplasts. Bacterial cells and fully intact plant cells require their own lysis protocol.

Once you have extracted your cell lysate containing your soluble, recombinant protein of choice/ have your membrane protein solubilisate at hand, PureCube, HiCap, and HighSpec Affinity MagBeads can be used to extract tagged, recombinant proteins with ultra-high affinity.

Please note that different affinity tags require different buffer components, e.g., His-tagged proteins are eluted by increasing concentrations of Imidazole, while antibody-based purifications use a specific peptide to elute your recombinant protein of choice.

You can download the protocols for the different purification buffers under these links:

His-tag	https://cube-biotech.com/media/59/90/af/1665052047/His-MagBeads%20Native.pdf
GST-tag	https://cube-biotech.com/media/63/28/f3/1666875446/Glutathione%20MAgBE-ads%20Purification%20Protocol.pdf
Strep-tag®	https://cube-biotech.com/media/67/8f/3d/1667387993/Strep-tag%20MagBeads%20purification%20protocol.pdf
Rho1D4-tag	https://cube-biotech.com/media/c9/f4/b2/1666947796/Rho1D4-tag%20MagBe-ads%20Purification%20Protocol.pdf

Affinity MagBeads have varying binding capacities and come in different suspension concentrations (5%-25%) (See Table 1). Please adjust the volume of MagBeads accordingly.

Table 1: Binding capacity of different MagBeads

Cube Biotech MagBead Types	Binding capacity of 1 ml pure beads (eg., 4 ml of 25% solution/20 ml of 5% solution
PureCube HiCap Strep-Tactin®	>7 mg/ml
PureCube Co-NTA	>30 mg/ml
PureCube Cu-NTA	>80 mg/ml
PureCube Ni-IDA	>70 mg/ml
PureCube Ni-INDIGO	>100 mg/ml
PureCube Ni-NTA	>80 mg/ml
PureCube Zn-NTA	>50 mg/ml
PureCube Glutathione	>20 mg/ml
HighSpec Rho1D4	>3 mg/ml

Evaluate the needed volume of MagBeads solution to extract the maximum amount of your recombinant protein. Keep in mind that pH, buffer composition, additives, tag availability, and other factors can dramatically influence the binding capacity and, therefore, the performance of the affinity resin. It is advised to perform pre-experiments to determine the optimal MagBead to Lysate/ Solubilizate ratio.

Protocol

1. Resuspend the MagBeads by vortexing and/or inverting.
2. Pipette the amount of choice, e.g., 4ml 25% solution (1ml pure MagBeads), into a container of choice, e.g., a 50 ml Falcon tube.
3. Place the container on the Cube L-shaped magnet and wait until all MagBeads are pulled to the magnetic surface and the supernatant remains fully clear (approx. 30s to 1 min). Make sure no MagBeads stick to the surface of the container.
4. Discard the supernatant by pipetting.
5. Add 5 to 10-fold buffer of choice (lysis buffer or washing buffer) to the MagBeads and mix by inoculation until fully mixed.
6. Place the container back on the magnet and wait until supernatant is fully clear (approx. 30s to 1 min).
7. Discard the supernatant by pipetting.

8. Repeat steps 5 -7 one more time to wash away all storage buffers from the MagBeads.

9. Now, your MagBeads are ready to be used in your Lysate/Solubilizate. Add the washed MagBeads to your Lysate/Solubilizate by pipetting.

10. Incubate the Lysate/Solubilizate – MagBead Mix for at least 1h (His-tagged proteins)/3h (Antibody-fused MagBeads) or overnight.

> **Important:** To ensure a proper binding, the mixture needs to be moving during incubation time (e.g., via a lab shaker). Do not use a magnetic stirrer, the beads will adhere to it.
> If the mixture is not moved during incubation time, the MagBeads will settle, and there will be no efficient target binding!

11. After incubation, the container is placed back onto the magnet. Depending on the volume of the container, settling the beads can take up to 10 min. For fast separation, we advise using smaller transfer containers, e.g., 500 ml for total volumes > 1 l. This way, the separation takes place in 1-3 minutes.

12. After removal of the supernatant, the beads can be transferred to a smaller container, e.g., a 50 ml Falcon Tube, for washing. Use a buffer tailored to your purification resin and make sure no MagBeads are lost during transfer steps.

13. Perform at least 5 washing steps. Add 5 to 10-fold washing buffer (e.g., 10 ml for 1 ml pure MagBeads) to the MagBeads and mix by inoculation until fully mixed.

14. Place the container back on the magnet and wait until the supernatant is fully clear (approx. 30s to 1 min).

15. Discard the supernatant by pipetting.

16. If you wish, The MagBeads can now be transferred to a smaller container for target protein elution.

17. To elute your target protein, perform at least three elution steps of 30 min to 1 h using a minimum of 50% pure MagBead volume (e.g., 500µl for 1 ml pure MagBeads). To ensure an efficient elution, the mixture needs to be moving during incubation (e.g., on a lab roller). If the mixture is not moving, the elution is not as efficient. If you cannot constantly mix the sample, inoculate every 5-10 minutes.

18. Place the container back on the magnet, wait until MagBeads have fully settled, and remove your eluate via pipetting.

19. Protein concentration can be measured via UV-Vis at 280nm, BSA standard, Bradford Assay, or a method of your choice. Make sure also to test the quality of your sample via SDS-PAGE and, if possible, via Western-Blot. A Size Exclusion Chromatography is advised to increase sample quality.