GFP-nAb™ Magnetic Agarose beads

Contents:

Store at 4ºC for up to 1 year, **do not freeze:**

GFP-nAb™ Magnetic Agarose beads (50% slurry) in PBS pH 7.4 with 20% ethanol.
Binding capacity - minimum 4µg EGFP per 10µl of slurry

Additional materials needed:

1.5ml microcentrifuge tubes
Phosphate Buffered Saline (PBS)
Protease inhibitor cocktail (optional)
EDTA (optional)
Magnetic stand for 1.5ml microcentrifuge tubes

Protocol – Magnetic Agarose Beads:

See **Buffer Recipes** for recommended buffers and **Notes** for important considerations for optimizing the protocol to your particular sample.

1. Harvest and wash cells

Add 1ml ice-cold PBS to a 10cm dish of cultured cells (~10^7 cells) expressing GFP fusion protein. Scrape cells from dish and transfer to a 1.5ml microcentrifuge tube on ice. Spin down at 500 x *g* for 3 minutes at 4ºC. Carefully remove and discard supernatant.

Gently resuspend cells in 1ml ice-cold PBS, then spin down at 500 x *g* for 3min at 4ºC, and carefully discard supernatant. Repeat wash once more.

2. Lyse cells

Resuspend the washed cell pellet in ~200µl ice-cold 1X Lysis Buffer (supplemented with protease inhibitor cocktail, see **Notes**) by pipetting up and down thoroughly. Incubate on ice for 30 minutes, pipetting up and down vigorously every 5-10 minutes.

Spin down at 20,000 x *g* (or max speed on a microcentrifuge) for 10 minutes at 4ºC. Transfer the supernatant to a new tube on ice.

Dilute the cell lysate with 1X Binding Buffer to a final volume of 500-1000µl.

3. Equilibrate beads

Resuspend the GFP-nAb™ Magnetic Agarose slurry by vortexing for 30 seconds. Immediately pipette 25µl of slurry into a 1.5ml microcentrifuge tube, followed by 500µl 1X Binding Buffer. Place on a magnetic stand to collect beads.

When the solution has fully cleared, carefully remove the supernatant by pipetting while the tube remains in contact with the stand.

Resuspend the beads in 500µl 1X Binding Buffer, vortex for 30 sec, then collect on magnetic stand and remove the supernatant. Repeat this wash once more.

4. Incubate with lysate

Add up to 700µl of diluted lysate to the washed beads, cap the tube, and gently vortex until the resin is fully resuspended. Tumble end-over-end for 10 min to 2 hours at 4ºC (or room temperature if your GFP fusion protein is known to be stable, see **Notes**).

5. Wash resin

Place the tube on a magnetic stand to collect beads. When the solution has fully cleared, carefully remove the supernatant by pipetting, transfer to a new tube, and save for analysis.

Resuspend the beads in 500µl 1X Binding Buffer, vortex for 30 sec, then place back on the magnetic stand. Carefully remove and discard the supernatant.

Resuspend the resin in 500µl 1X Wash Buffer, vortex for 30 sec, then place back on the magnetic stand. Carefully remove and discard the supernatant. Repeat this wash once more.

6. Elute GFP-tagged protein

Resuspend the resin in 50µl of 1X Elution Buffer, then gently pipette up and down for 30 seconds, making sure that all of the GFP-nAb™ beads are resuspended.

Place on the magnetic stand. Very carefully remove the supernatant, taking care not to disturb the beads, and transfer into a fresh tube containing 5µl of 1X Neutralization Buffer.

Repeat if desired, with an additional 50µl of 1X Elution Buffer, adding 5µl 1X Neutralization buffer to the supernatant after pooling with the first elution.

Notes:

The binding capacity of each lot of GFP-nAb™ Magnetic Agarose beads is assayed using purified EGFP, and may be higher or lower for a given GFP fusion protein depending on the molecular weight and the three-dimensional conformation of the fusion. To decrease the likelihood of steric hindrance of the interaction between GFP and GFP-nAb™, we recommend using long, flexible linker sequences (such as 5x(Gly-Gly-Ser)) between GFP and the fusion partner.

GFP-nAb™ Magnetic Agarose beads have a very high binding affinity for GFP, and shorter incubation times (~10 minutes) are sufficient to achieve complete binding of most GFP fusion proteins from cell lysates. However, particular GFP fusions may display diminished affinity if there is steric hindrance of the interaction between the antibody and the GFP tag (see above). Proteins present at very low concentrations or with lower affinities may require longer incubation times, up to 2 hours, to achieve maximal recovery. We recommend empirical determination of optimal binding time for each new GFP fusion.

Buffers other than the recommended ones may be used if desired. GFP-nAb™ binding affinity is essentially unchanged in Tris, phosphate, HEPES, MOPS, and many other biological buffers between pH 7-9, containing up to 2M NaCl, and with many standard cell lysis buffers (including commercial reagents; see below for detergent concentration considerations). If desired, urea may be added to lysis, binding, and wash buffers in cases where extremely stringent washing is desired. The binding capacity of GFP-nAb is not changed by addition of up to 2M urea in all buffers.

Cell harvest and lysis should be performed with ice-cold buffers. Although optional, we strongly recommend adding a protease inhibitor cocktail to the Lysis Buffer to prevent degradation of your target mNG fusion or its native binding partners. ***If desired, reducing agents (up to 1mM DTT) may be added to all buffers.****

To avoid non-specific binding to the GFP-nAb™ Magnetic Agarose matrix, the detergent concentration in diluted cell lysate should not exceed 0.2%. At 1X, the provided Lysis Buffer contains 0.5% detergent, and so the lysate must be diluted at least 1:2.5 prior to binding. GFP fusion binding to GFP-nAb™ Magnetic Agarose beads will not be diminished by diluting the lysate.

For many proteins, it is possible to perform most steps at room temperature. However, the stability of any particular GFP fusion in cell lysate must be determined empirically. If your GFP fusion protein or its native binding partners are especially susceptible to proteolysis, we strongly recommend performing the entire protocol at 4ºC, with all buffers chilled on ice prior to beginning. As an additional precaution, Binding and Wash buffers may be supplemented with 0.5mM EDTA and/or protease inhibitors, if desired.

Before pipetting the desired volume of GFP-nAb™ Magnetic Agarose slurry, it is important to thoroughly resuspend it by vortexing. Pipette the slurry immediately after resuspending, before the beads have started to settle. For each protocol step requiring resuspension of the beads, ensure that they are vortexed thoroughly and that no clumps remain.

The use of glycine buffer, pH 2.5, followed by immediate neutralization, is recommended for elution of GFP fusion proteins from GFP-nAb™ Magnetic Agarose beads. This method is faster and gentler than boiling, and can allow recovery of natively folded protein (e.g. for enzyme assays).

*** -Updated information***

Buffer Recipes:

1X Lysis Buffer

20mM Tris-HCl pH 7.5
150mM NaCl
1mM EDTA
0.5% NP-40

1X Binding Buffer

10mM Tris-HCl pH 7.5
150mM NaCl

1X Wash Buffer

10mM Tris-HCl pH 7.5
500mM NaCl

1X Elution Buffer

200mM glycine pH 2.5

1x Neutralization Buffer

1M Tris Base