



GFP-nAb Agarose resin

Contents:

Store at 4°C for up to 1 year, **do not freeze**:
GFP-nAb™ Agarose (50% slurry)
Binding capacity - minimum 5ug EGFP per 10ul of slurry
Average bead size ~90um

Protocol -- Spin Columns (Recommended):

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 (~10⁷ 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 4°C. Carefully remove and discard supernatant.

Gently resuspend cells in 1ml ice-cold PBS, then spin down at 500 x *g* for 3min at 4°C, 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 4°C. 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 resin*

Snap the end from the bottom of a new spin column and place it in a 2ml microcentrifuge tube. Resuspend the GFP-nAb™ Agarose slurry by flicking the tube or gently vortexing. Immediately pipette 25µl of slurry (using a wide-orifice 200µl pipette tip or standard 1000µl tip) into the spin column, followed by 500µl 1X Binding Buffer. Spin down at 100x *g* for 5-10 seconds.

Discard the flow-through and add 500µl 1X Binding Buffer to the spin column, then spin down at 100 x *g* for 5-10 sec. Repeat this wash once more, then remove the spin column from the collection tube and secure the bottom cap to the spin column.

4. Incubate with lysate

Add up to 700µl of diluted lysate to the spin column, securely cap the top, and invert/flick until the resin is fully resuspended. Tumble end-over-end for 10 min to 2 hours at 4°C (or room temperature if your GFP fusion protein is known to be stable, see **Notes**).

5. Wash resin*

Remove the bottom cap from the spin column and place it in a new collection tube. Spin down at 100 x g for 5-10 sec. Transfer the flow-through to a new tube and save for analysis. Place the spin column into a new collection tube and remove the top cap.

Add 500µl 1X Binding Buffer to the spin column and spin down at 100 x g for 5-10 sec. Discard the flow-through.

Add 500µl 1X Wash Buffer to the spin column and spin down at 100 x g for 5-10 sec. Discard flow-through.

Place a new bottom cap on the spin column.

6. Elute GFP-tagged protein

Pipette 50µl of 1X Elution Buffer into the spin column and gently pipette up and down for 30 seconds, making sure that all of the GFP-nAb™ resin is resuspended.

Remove the bottom cap and place the spin column into a fresh collection tube containing 5µl of 1X Neutralization Buffer. *A small amount of buffer containing eluted protein may have collected in the bottom cap -- to maximize recovery, pipet this buffer and add it to the collection tube before spinning down the column.*

Recover eluted protein by spinning down the column at 1000 x g for 30-60 sec.

Repeat if desired, using a new collection tube, then pool the eluted protein for analysis.

Tube Protocol next page.

Tube Protocol:

Harvest and lyse cells following steps 1 and 2 for **Spin Column Protocol**.

3. Equilibrate resin

Resuspend the GFP-nAb™ Agarose slurry by flicking the tube or gently vortexing. Immediately pipette 25µl of slurry (using a wide-orifice 200µl pipette tip or standard 1000µl tip) a 1.5ml microcentrifuge tube, followed by 500µl 1X Binding Buffer. Spin down at 1000 x *g* for 30-60 seconds.

Carefully remove supernatant by pipetting and discard, taking care not to disturb the GFP-nAb™ resin. Resuspend the resin in 500µl 1X Binding Buffer, then spin down at 1000 x *g* for 30-60 sec. Repeat this wash once more.

4. Incubate with lysate

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

5. Wash resin

Spin down the tube at 1000 x *g* for 30-60 sec to pellet the resin. Carefully remove the supernatant by pipetting, transfer to a new tube, and save for analysis.

Resuspend the resin in 500µl 1X Binding Buffer, then spin down at 1000 x *g* for 30-60 sec. Carefully remove and discard the supernatant.

Resuspend the resin in 500µl 1X Wash Buffer, then spin down at 1000 x *g* for 30-60 sec. 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™ resin is resuspended.

Spin down the tube at 1000 x *g* for 30-60 sec. Very carefully remove the supernatant, taking care not to disturb the resin, 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 **Spin Column Protocol** is highly recommended for general use, as it maximizes recovery of eluted protein while eliminating losses from accidental pipetting of GFP-nAb™ resin in tubes, as well as contamination from incomplete washing due to over-cautious removal of supernatant. After cell harvest and lysis, the Spin Column Protocol can be completed in under 20 minutes with multiple samples. The 20-reaction GFP-nAb™ Spin Kit provides all necessary buffers and spin columns for your convenience.

The binding capacity of each lot of GFP-nAb™ Agarose resin 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™ Agarose has 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 GFP fusion or its native binding partners. ***If desired, reducing agents may be added to all buffers. Incubation with up to 50mM DTT does not cause a measurable decrease in binding capacity.****

Spin steps may be performed by quickly pulsing to ~100 x g (5 - 10 sec). Longer spins at higher speeds are acceptable (up to 1000 x g for 30 - 60 sec), but may lead to some reduction in binding capacity and sample elution. In experiments in which sample quantity is limiting, it is advisable to avoid over-drying of the resin, using short spin times and proceeding to the next protocol step immediately after spinning. Allowing the resin to dry at room temperature for 10 minutes after the final wash step and spin leads to a ~50% reduction in elution efficiency. In all cases, the final elution spin should be performed at 1000 x g for 30 - 60 sec to maximize recovery.*

To avoid non-specific binding to the GFP-nAb™ 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™ Agarose 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 4°C, 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™ Agarose slurry, it is important to thoroughly resuspend it by flicking the tube or gently vortexing. Pipette the slurry immediately after resuspending, before the resin has started to settle. The slurry is best pipetted using standard 1000µl tips or wide-orifice 200µl tips.

The use of glycine buffer, pH 2.5, followed by immediate neutralization, is recommended for elution of GFP fusion proteins from GFP-nAb™ Agarose. 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

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