Allele
mNG-nAb™

mNG-nAb™ Agarose Spin Kit (20 rxn)

Contents:

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

mNG-nAb™ Agarose (50% slurry)	500µl
10X Lysis Buffer	500µl
20X Binding Buffer	5ml
10X Wash Buffer	1.2ml
5X Elution Buffer	500µl
1X Neutralization Buffer	300µl

Store at room temperature:

Spin columns	20
Top caps	20
Bottom caps	40

Additional materials needed:

1.5ml microcentrifuge tubes
2ml microcentrifuge tubes
Phosphate Buffered Saline (PBS)
Protease inhibitor cocktail (optional)
EDTA (optional)

Protocol:

See **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 mNG 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 resin**

Snap the end from the bottom of a new spin column and place it in a 2ml microcentrifuge tube. Resuspend the mNG-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 100 x *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 4ºC (or room temperature if your mNG 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 mNG-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 mNG-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.

Notes:

The binding capacity of each lot of mNG-nAb™ Agarose resin is assayed using purified mNG, and may be higher or lower for a given mNG 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 mNG and mNG-nAb™, we recommend using long, flexible linker sequences (such as 5x(Gly-Gly-Ser)) between mNG and the fusion partner.

mNG-nAb™ Agarose has a very high binding affinity for mNG, and shorter incubation times (~10 minutes) are sufficient to achieve complete binding of most mNG fusion proteins from cell lysates. However, particular mNG fusions may display diminished affinity if there is steric hindrance of the interaction between the antibody and the mNG 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 mNG fusion.

Buffers other than the recommended ones may be used if desired. mNG-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 mNG-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 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 mNG-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. mNG fusion binding to mNG-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 mNG fusion in cell lysate must be determined empirically. If your mNG 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 mNG-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 mNG fusion proteins from mNG-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 compositions:

10X Lysis Buffer

200mM Tris-HCl pH 7.5
1.5M NaCl
10mM EDTA
5% NP-40

20X Binding Buffer

200mM Tris-HCl pH 7.5
3M NaCl

10X Wash Buffer

100mM Tris-HCl pH 7.5
5M NaCl

5X Elution Buffer

1M glycine pH 2.5

1x Neutralization Buffer

1M Tris Base

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