CoIP with nAb

Background

Co-immunoprecipitation (CoIP) is one of the most commonly used techniques for studying protein-protein interactions. A 'bait' protein is precipitated out of solution using an antibody, and any interacting 'target' proteins will also be isolated in the process. The success of this technique is dependent on three requirements being fulfilled, you must have an antibody against your bait protein, it must be highly specific to limit background, and this antibody must be coupled to a solid substrate.

It can be exceedingly difficult to find an appropriate antibody against specific proteins that fulfill the requirements for a successful *in vivo* CoIP assay. A common method employed by researchers is the use of a common affinity tag (i.e. GFP) fused to their 'bait' protein. nAbs are highly specific antibodies that enables complete complete sample pulldown with no background or off target binding. They display a high degree of stability, permitting their use in a variety of cell lysates and buffer conditions. All nAbs are available pre-conjugated to agarose resin and are ready for direct use in CoIP reactions.

Materials

- nAb™ agarose or nAb™ magnetic resin
- nAb™ agarose control or nAb™ magnetic control
- Cells expressing protein(s) of interest
- Lysis Buffer [20mM Tris-HCl pH 7.5,150mM NaCl,1mM EDTA,0.5% NP-40]
- Binding Buffer [10mM Tris-HCl pH 7.5, 150mM NaCl]
- Wash Buffer [10mM Tris-HCl pH 7.5, 500mM NaCl]
- Elution Buffer [200mM glycine pH 2.5]
- Neutralization Buffer [1M Tris Base]

Equipment

- Microcentrifuge Tubes
- Microcentrifuge
- Spin Columns
- Micropipettor with tips
- Rotator

Protocol

Note: This protocol is specifically written for the GFP-nAb™ Agarose Spin Kit or GFP-nAb™ Agarose Resin. For a nAb™ Magnetic Resin protocol visit www.allelebiotech.com/nab

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 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 4ºC (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.

Eluted materials are ready for use in Wester Blot analysis, Mass Spectrometry or any additional down steam analysis.