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Immunoprecipitation (IP)

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nguyen.tha[1]

[1]Laboratory of Michael Lazarou, Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia

ASAP workspace

nguyen.tha

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol details about immunoprecipitation using anti-HA magnetic beads.

Attachments

DOCX

635-1314.docx

17KB

Materials

Buffers and reagents:

- **IP base buffer**: [M] 50 millimolar (mM) Tris-Cl (pH 7.5 when cold), [M] 150 millimolar (mM) NaCl
- **Bead equilibration buffer**: IP base buffer supplemented with 0.1% Tween20
- **IP wash buffer**: IP base buffer supplemented with 0.1% TX-100 and 1x cOmplete, EDTA-free protease inhibitor cocktail
- Pierce™ Anti-HA Magnetic Beads **Thermo Fisher Catalog #**88836
- Benzonase® Nuclease **Merck Millipore (EMD Millipore) Catalog #**E1014-25KU
- cOmplete™ EDTA-free Protease Inhibitor Cocktail **Roche Catalog #**4693132001
- **Elution buffer**: NuPAGE™ LDS Sample Buffer (4X) **Thermo Fisher Scientific Catalog #**NP0007

Note

Elution buffer can be aliquoted and stored at -20 °C or -80 °C .

Troubleshooting

Procedures

3h 50m

1 Lyse cell pellets (5-7 mg) in 500 µL IP lysis buffer containing IP base buffer supplemented with 1x cOmplete, EDTA-free protease inhibitor cocktail and 0.1 µL of benzonase and incubate samples On ice for 00:30:00 . Mix the sample by inverting the eppies gently every 5 min. 30m

2 Wash anti-HA beads with 500 µL of bead equilibration buffer.

3 Repeat step 2 twice.

4 Centrifuge the cell lysates at max speed for 00:10:00 at 4 °C . 10m

5 Carefully transfer cleared lysates into 2 ml eppies and take 50 µL from each tube for “Input” samples.

6 Gently add 1000 µL of IP base buffer containing 1x cOmplete, EDTA-free protease inhibitor cocktail to the rest of each sample to dilute out the detergent.

7 Incubated the diluted cleared lysates with the anti-HA magenetic beads on a rotary mixer for 03:00:00 at 4 °C . 3h

8 Collect beads on a magnetic rack and aspirate the unbounds.

9 Wash with 1 mL IP wash buffer.

10 Repeat steps 7-8 another 4 times.

Note

For the last wash, make sure to remove all the liquid off the beads.

11 Elute with 25 µL elution buffer by boiling at shaking at 99 °C for 00:10:00 .

10m