

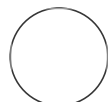


FEB 23, 2023

Immunoprecipitation (IP)

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ABSTRACT

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

ATTACHMENTS

[635-1314.docx](#)

OPEN ACCESS

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

Created: Feb 07, 2023





Last Modified: Feb 23, 2023

PROTOCOL integer ID:
76523



Keywords:
immunoprecipitation

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 #E10125KU**
-  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 .

Procedures

3h 50m

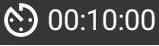

- 1 Lyse cell pellets ( 5-7 mg) in  500 µL IP lysis buffer containing IP base buffer supplemented with 1x cOmplete, EDTA-free protease inhibitor cocktail and  0.1 µL of benzonase and incubate samples  On ice for  00:30:00 . Mix the sample by inverting the eppies gently every 5 min. 30m



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




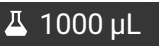
3 Repeat step 2 twice.

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

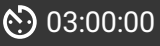

10m



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 magnetic 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