Immunoprecipitation

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Abstract

This protocol is suitable for both endogeneous and overexpressed protein immunoprecipitations.

Citation: Vivian Liu Immunoprecipitation. **protocols.io**
dx.doi.org/10.17504/protocols.io.dkm4u5
Published: 05 Aug 2015

Protocol

Step 1.
Take cell dishes out of incubator and pre-cool on ice.

NOTES
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Step 1~ 7 are for IP lysate preparation.

Step 2.
Rinse cells gently with ice-cold PBS for twice.

REAGENTS
1X PBS (Phosphate-buffered saline) by Contributed by users

Step 3.
Scrape cells and spin down @ 500 rpm, 10min, 4 °C.

Step 4.
Add 500l ul IP lysis buffer to each cell pellets (upto 2E+7 cells).

NOTES
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Lysis Buffer:
TrisHCl (PH8.0) 20mM
NaCl 300mM
EDTA 0.5mM
Na3VO4 1mM
NaF 50mM
NP-40 1%
TritonX-100 1%
Add protease inhibitors before using.
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IP lysis buffer recipe

Step 5.
Rotate for 2h at 4°C. Centrifuge 14,000rpm for 30 min. Save supernatant.

Step 6.
(Optional) Roughly determine protein concentration with OD280. Adjust all samples to the same

concentration.

Step 7.
Determine amount of beads needed (Rockland True Blot IP beads; 20 ul beads per 500ul sample).

Step 8.
Wash beads in 1ml lysis buffer for twice. Spin down @500g, 30s.

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Step 8~11 are for IP beads preparation.

Step 9.
Resuspend pelleted beads in 1mg/ml BSA , rotate @ RT, 10min.

Step 10.
Remove BSA and wash beads in lysis buffer for 3 times.

Step 11.
Resuspend pelleted beads 1:1 with lysis buffer.

Step 12.
Save 40ul of IP lysate per sample as input.

NOTES
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Step 12~16 are for the IP process.

Step 13.
Add primary Ab and beads to IP lysate.

Step 14.
Rotate @4 degree for overnight.

Step 15.
Pellet beads @ 500g for 30sec @RT and discard the supernatant.

Step 16.
Wash beads in 1ml ice-cold lysis buffer. Repeat 3 times.

Step 17.
Resuspend beads in 30ul of 2× loading buffer.

NOTES
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Step 17~20 are for SDS-PAGE sample preparation.

Step 18.
Boil the beads for 5min.

Step 19.
Centrifuge @ RT for 1min.

Step 20.
Save supernatant and perform SDS-PAGE.

NOTES
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WELL DONE & GOOD LUCK!