Immunoprecipitation using Protein A/G Magnetic Beads

New England Biolabs

Abstract

This is the protocol for immunoprecipitation using Protein A/G Magnetic Beads.

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Before start

Use 25 µl of Protein A/G Magnetic Beads per 200 µl of crude cell lysate containing 200-500 µg of total protein in a standard immunoprecipitation protocol. It is important to increase the volume of beads proportionately for larger cell lysate volumes.

Materials

- Protein A Magnetic Beads - 1 ml S1425S by New England Biolabs
- Protein G Magnetic Beads - 1 ml S1430S by New England Biolabs

Protocol

Cell Lysis

Step 1.
Rinse a 60 mm culture dish of confluent cells with PBS

Cell Lysis

Step 2.
Lyse the cells with 0.5 ml cold Immunoprecipitation Buffer

AMOUNT
1 ml Additional info:

PROTOCOL

. NEB Immunoprecip. Buffer

CONTACT: New England Biolabs
Step 2.1.
50 mM NaCl

Step 2.2.
10 mM Tris-HCl (pH 7.4)

Step 2.3.

1 mM EDTA

Step 2.4.
1 mM EGTA (pH 8.0)

Step 2.5.
0.2 mM sodium ortho-vanadate

Step 2.6.
0.2 mM PMSF

Step 2.7.
1% Triton X-100

Step 2.8.
0.5% NP-40

Cell Lysis

Step 3.
Maintain constant agitation for 30 minutes at 4°C

DURATION
00:30:00

Cell Lysis

Step 4.
Scrape the cells from the dish

Cell Lysis

Step 5.
Sonicate on ice for 5 seconds; repeat 4 times

Cell Lysis

Step 6.
Centrifuge for 5 minutes at 4°C

DURATION
00:05:00

Cell Lysis

Step 7.
Assay for total protein then adjust concentration to approximately 1 mg/ml with Immunoprecipitation Buffer

NOTES
New England Biolabs 31 Jan 2015
The supernatant is the crude cell lysate.

Immunoprecipitation

Step 8.
In a 1.5 ml microcentrifuge tube, add **25 µl** Protein A/G Magnetic Beads to **200 µl** of crude cell extract

NOTES
New England Biolabs 01 Feb 2015
Steps 8-12 pre-clear crude cell extract of proteins which can bind non-specifically to the beads

Immunoprecipitation

Step 9.
Gently vortex

Immunoprecipitation

Step 10.
Incubate at 4°C for 1 hour

DURATION
01:00:00

Immunoprecipitation

Step 11.
Apply magnetic field for 30 seconds to pull beads to the side of the tube

DURATION
00:00:30

Immunoprecipitation

Step 12.
Pipette supernatant to a clean 1.5 ml microcentrifuge tube and discard the beads.

Immunoprecipitation

Step 13.
Add 1-5 µg of antibody to crude cell lysate

Immunoprecipitation

Step 14.
Vortex

Immunoprecipitation

Step 15.
Incubate at 4°C for 1 hour

DURATION
01:00:00

NOTES
New England Biolabs 01 Feb 2015
If monoclonal antibodies are used, add 5 µg rabbit anti-mouse IgG antibody. Vortex and incubate an additional 30 minutes at 4°C. Alternatively, Protein G Magnetic Beads (NEB #S1430S) can be used for immunoprecipitations with monoclonal antibodies.

Immunoprecipitation

Step 16.
Add **25 µl** of Protein A/G Magnetic Beads suspension

Immunoprecipitation

Step 17.
Gently vortex

Immunoprecipitation

Step 18.
Incubate with agitation for 1 hour at 4°C.

DURATION
01:00:00

Immunoprecipitation

Step 19.
Apply magnetic field to pull beads to the side of the tube

Immunoprecipitation

Step 20.
Carefully pipette to remove supernatant

Immunoprecipitation

Step 21.
(wash #1) Wash with **500 µl** of Immunoprecipitation Buffer by gentle vortex

Immunoprecipitation

Step 22.
(wash #1) Apply magnetic field then remove supernatant and discard

Immunoprecipitation

Step 23.
(wash #2) Wash with **500 µl** of Immunoprecipitation Buffer by gentle vortex

Immunoprecipitation

Step 24.
(wash #2) Apply magnetic field then remove supernatant and discard

Immunoprecipitation

Step 25.
(wash #3) Wash with **500 µl** of Immunoprecipitation Buffer by gentle vortex

Immunoprecipitation

Step 26.
(wash #3) Apply magnetic field then remove supernatant and discard

Immunoprecipitation

Step 27.
Resuspend bead pellet in **30 µl** of 3X SDS Sample Loading Buffer

AMOUNT
30 µl Additional info:
DURATION
00:05:00

PROTOCOL

. NEB 3X SDS Sample Loading Buffer

CONTACT: New England Biolabs
Step 27.1.
187.5 mM Tris-HCl (pH 6.8)

Step 27.2.
6% (w/v) SDS

Step 27.3.
30% glycerol

Step 27.4.
150 mM DTT

Step 27.5.
0.03% (w/v) bromophenol blue

Step 27.6.
2% β-mercaptoethanol

Immunoprecipitation

Step 28.
Incubate sample at 70°C for 5 minutes.

DURATION

00:05:00

Immunoprecipitation

Step 29.

Apply magnetic field to sample then load supernatant on SDS-PAGE gel and electrophorese.