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Immunoprecipitation using Protein A/G Magnetic Beads V.2

New England Biolabs[1]

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This protocol explains immunoprecipitation using Protein A/G Magnetic Beads.

DOI

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https://www.neb.com/protocols/0001/01/01/immunoprecipitation-using-protein-ag-magnetic-beads

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Protein A magnetic beads, Protein G magnetic beads, immunoprecipitating, non-specific binding to beads, pre-clearing crude cell extract of proteins, Immunoprecipitation, IP

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MATERIALS

Sodium Orthovanadate (Vanadate) - 1 ml **New England Biolabs Catalog #P0758S**

Protein A Magnetic Beads - 1 ml **New England Biolabs Catalog #S1425S**

Protein G Magnetic Beads - 1 ml **New England Biolabs Catalog #S1430S**

PMSF **Sigma Aldrich Catalog #P7626**

Bromophenol blue **Bio Basic Inc. Catalog #BB2230.SIZE.25g**

Glycerol **Bio Basic Inc. Catalog #GB0232.SIZE.500ml**

SDS **Bio Basic Inc. Catalog #SB0485.SIZE.100g**

DTT (Dithiothreitol) (> 99% pure) Protease free **Gold Biotechnology Catalog #DTT**

EGTA **Gold Biotechnology Catalog #E-217**

Triton X-100 **Sigma Catalog #93426**

Tris-HCl **Life Technologies Catalog #AM9855**

2-Mercaptoethanol **Sigma Aldrich Catalog #M3148**

EDTA **Fisher Scientific Catalog #16 004Y**

Immunoprecipitation Buffer:

A	B
NaCl	150 mM
Tris-HCl (pH 7.4)	10 mM
EDTA	1 mM
EGTA (pH 8.0)	1 mM
Sodium ortho-vanadate	0.2 mM
PMSF	0.2 mM
Triton X-100	1%
NP-40	0.50%

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3X SDS Sample Loading Buffer:

A	B
Tris-HCl (pH 6.8)	187.5 mM
SDS	6%(w/v)
Glycerol	30%
DTT	150 mM
Bromophenol blue	0.03% (w/v)
β-mercaptoethanol)	2%

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

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.

Prepare *Immunoprecipitation buffer* with the following reagents:

A	B
NaCl	150 mM
Tris-HCl (pH 7.4)	10 mM
EDTA	1 mM
EGTA (pH 8.0)	1 mM
Sodium ortho-vanadate	0.2 mM
PMSF	0.2 mM
Triton X-100	1%
NP-40	0.50%

Prepare *3X SDS Sample Loading Buffer* using the following reagents:

A	B
Tris-HCl (pH 6.8)	187.5 mM
SDS	6%(w/v)
Glycerol	30%
DTT	150 mM
Bromophenol blue	0.03% (w/v)
β-mercaptoethanol)	2%

Cell Lysis

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

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2 Lyse the cells with **0.5 mL cold Immunoprecipitation Buffer** .

Immunoprecipitation buffer is prepared with the following reagents:

A	B
NaCl	150 mM
Tris-HCl (pH 7.4)	10 mM
EDTA	1 mM
EGTA (pH 8.0)	1 mM
Sodium ortho-vanadate	0.2 mM
PMSF	0.2 mM
Triton X-100	1%
NP-40	0.50%

3 Maintain constant agitation for **00:30:00** at **4 °C** .

4 Scrape the cells from the dish.

5 Sonicate **On ice** for **00:00:05** ; repeat 4 more times:

5.1 Sonicate **On ice** for **00:00:05** (1/4).

5.2 Sonicate **On ice** for **00:00:05** (2/4).

5.3 Sonicate **On ice** for **00:00:05** (3/4).

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5.4 Sonicate **On ice** for **00:00:05** (4/4).

6

Centrifuge for **00:05:00** at **4 °C** .

7

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

The supernatant is the crude cell lysate.

Immunoprecipitation

8

In a 1.5 ml microcentrifuge tube, add **25 µL protein A/G Magnetic Beads** to **200 µL crude cell extract** .

Steps 8-12 pre-clear crude cell extract of proteins which can bind non-specifically to the beads.

9 Gently vortex.

10

Incubate at **4 °C** for **01:00:00** .

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11 Apply magnetic field for **00:00:30** to pull beads to the side of the tube.

12 

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

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

14 Vortex.

15 

Incubate at **4 °C** for **01:00:00** .

If monoclonal antibodies are used, add **5 µg rabbit anti-mouse IgG antibody** .

Vortex and incubate an additional **00:30:00** at **4 °C** . Alternatively, Protein G Magnetic Beads (NEB #S1430S) can be used for immunoprecipitations with monoclonal antibodies.

16 

Add **25 µL Protein A/G Magnetic Beads suspension .**

17 Gently vortex.

18 

Incubate with agitation for **01:00:00** at **4 °C** .

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

20

Carefully pipette to remove supernatant.

21

Wash with **500 µL Immunoprecipitation Buffer** by gentle vortex.

22

Apply magnetic field, then remove supernatant and discard.

23

Repeat wash steps two more times:

23.1

Wash with **500 µL Immunoprecipitation Buffer** by gentle vortex. (1/2)

23.2

Apply magnetic field, then remove supernatant and discard. (1/2)

23.3

Wash with **500 µL Immunoprecipitation Buffer** by gentle vortex. (2/2)

23.4

Apply magnetic field, then remove supernatant and discard. (2/2)

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Resuspend bead pellet in **30 µL 3X SDS Sample Loading Buffer** .

3X SDS Sample Loading Buffer is prepared using the following reagents:

A	B
Tris-HCl (pH 6.8)	187.5 mM
SDS	6%(w/v)
Glycerol	30%
DTT	150 mM
Bromophenol blue	0.03% (w/v)
β-mercaptoethanol)	2%

25 

Incubate sample at **70 °C** for **00:05:00** .

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

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