Immunoprecipitation of protein lysate from culture cells using Src antibody

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Method Article

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Abstract

It is a procedure for immunoprecipitation using protein lysate from a neuronal cell line, NSC34, and a muscle cell line, C2C12 using Dynabeads™ Protein G Immunoprecipitation Kit (ThermoFisher).

Introduction

This protocol was used in our *Nature Communications* paper.

Reagents

CelLytic™ M (Sigma)

PBS-T

Dynabeads™ Protein G

Immunoprecipitation Kit (ThermoFisher)

Src antibody (ab16885)

Sample Buffer Solution (2ME+)(x4) (Wako)

Equipment

6 well plate

Cell scraper

1.5 ml tissue grinder

DynaMag™ Magnet (ThermoFisher)

1.5ml protein LoBind tube

Heat Block

Procedure

Preparation of lysates

1. Harvest NSC34 or C2C12 cells up to 80-100 % in 6 well plate
2. Aspirate media and wash plate with PBS

3. Aspirate PBS and add 500 µl of CelLytic™ M

4. Scrape cells with a cell scraper and collect media in a 1.5 ml tissue grinder

5. Preserve 10% of collected media at 4°C

Bind antibody

1. Vortex Dynabeads™ magnetic beads in the vial for 30 Sec

2. Transfer 50 µl of Dynabeads™ magnetic beads to a 1.5ml tube

3. Place the tube on the magnet to separate the beads from the solution, and remove the supernatant

4. Remove the tube from the magnet

5. Add 10 µg of antibody diluted in 200 µL of Ab Binding and Washing Buffer to the magnetic beads

6. Incubate with rotation for 30 minutes at room temperature

7. Place the tube on the magnet and remove the supernatant

8. Remove the tube from the magnet and resuspend the magnetic bead and antibody complex in 200 µL of PBS-T

9. Wash by gentle tapping

Immunoprecipitate target antigen

1. Place the tube on the magnet and remove the supernatant

2. Add the cell lysate to the tube and gently tap to resuspend the magnetic bead-Ab complex

3. Incubate with rotation for 60 minutes at room temperature

4. Place the tube on the magnet.

5. Wash the magnetic bead-Ab-Ag complex 3 times using 200 µL of Washing Buffer for each wash. Separate on the magnet between each wash, remove supernatant, and resuspend by gentle tapping.

6. Resuspend the magnetic bead-Ab-Ag complex in 100 µL Washing Buffer and transfer the bead suspension to a clean tube

Elute target antigen

1. Place the tube containing the magnetic bead-Ab-Ag complex on the magnet and remove the supernatant.

2. Add 30 µL of Elution Buffer and 10 µL of Sample Buffer Solution (2ME+)(x4) and gently tap

3. Heat for 10 min at 70ºC.

4. Place the tube on the magnet and load the sample onto a gel

Troubleshooting

Time Taken

Anticipated Results

References

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