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Immunoprecipitation with GFP-Trap Beads

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We use this protocol and it's working

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Abstract

A protocol to perform co-immunoprecipitation (CoIP) experiments with GFP tagged proteins.

Materials

Cell culture materials:
DMEM (Thermo Fisher Scientific, 11965-092)
FBS (Thermo Fisher Scientific, 16140-071)
Penicillin/Streptomicin (10,000 U/mL; Thermo Fisher Scientific, 15140122)
PBS (Thermo Fisher Scientific, 10010023)
Earle's Balanced Salt Solution (EBSS; Thermo Fisher Scientific, 24010043)

Transfection Reagents:
Opti-Mem (Thermo Fisher Scientific, 31985062)
Lipofectamine 3000 (Thermo Fisher Scientific, L3000008)

Lysis Buffer:
lysis buffer (10 mM Tris pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.5% NP-40) with Protease Inhibitor Cocktail
Tris (American Bio, AB02000-05000)
NaCl (Sigma-Aldrich, S9888-25G)
EDTA (Sigma-Aldrich, 3690)
Nonidet P40 Substitute (Roche, 11754599001)
COmplete mini EDTA Free (Roche, 11836170001)

Other reagents:
GFP-Trap beads (Proteintech, gtma-20)

Cell culture and treatments

10m

1 Culture the HEK293 cells at 37 °C in 5% CO_2 and DMEM containing 10% FBS, 1000 U/mL penicillin, a [M] 1 mg/mL streptomycin.

2 For any given experiment, plate the cells on 15 cm plates at such density so as to be approximately 95% confluent at the time of lysis.

3 If transfecting the sample, do so when the cells are approximately 70-80% confluent. Prepare the following reagents for transfection with lipofectamine 3000:

3.1 For transfected plasmids, each 15 cm plate requires 10 µg of each target DNA construct. Prepare a master mix consisting of 10 µg of DNA, 700 µL of opti-mem low serum medium, and 20 µL of P3000 reagent per well. Prepare an additional mix consisting of 700 µL of opti-mem and 20 µL of lipofectamine 3000 per well. Combine the DNA + P3000 and lipofectamine solutions together, and allow to incubate for 00:10:00 at Room temperature. 10m

Cell lysis and sample preparation

4 Supplement lysis buffer (10 mM Tris pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.5% NP-40) with Protease Inhibitor Cocktail (Roche) and chill On ice.

5 Aspirate media from cells, add PBS, and scrape cells using a cell scraper (Corning) On ice

6 Pipette the resuspended cells into Eppendorf tube.

7 Centrifuge at 500 x g, 4°C, 00:03:00, aspirate the excess PBS.

8 Pipette 150 uL lysis buffer onto cell pellet, pipette mixture up and down 10 times with a P-200 pipette tip

Note

NOTE: Take care not to introduce bubbles.

9 Incubate Eppendorf tube On ice for 00:05:00 .

10 Determine protein concentration in sample using Bradford Assay.

Immunoprecipitation

2h 10m 5s

11 Prepare GFP-Trap beads for the IP reaction:

11.1 Pipette 25 µL of the GFP-Trap bead slurry into eppendorf tubes for each reaction.

11.2 Pipette 500 µL of lysis buffer onto the bead slurry. Mix the tube by inverting five times.

11.3 Precipitate the beads using a magnetic rack. Aspirate the lysis buffer, including the buffer trapped at the top of the tube.

11.4 Pipette 500 µL of lysis buffer onto the bead slurry. Mix the tube by inverting five times.

11.5 Precipitate the beads using a magnetic rack. Aspirate the lysis buffer, including the buffer trapped at the top of the tube.

12 Pipette equal amounts of lysate (by protein level) onto the washed GFP-Trap beads. Adjust the volume up to 500 µL .

13 Incubate the samples at 4 °C for 02:00:00 , rotating. 2h

14 Perform the following washes at Room temperature :

14.1 Aspirate the flow through. Pipette 500 µL of lysis buffer onto the bead slurry. Mix the tube by inverting five times. Precipitate the beads using a magnetic rack. Aspirate the lysis buffer, including the buffer trapped at the top of the tube.

14.2 Pipette 500 µL of lysis buffer onto the bead slurry. Mix the tube by inverting five times. Precipitate the beads using a magnetic rack. Aspirate the lysis buffer, including the buffer trapped at the top of the tube.

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14.5 Pipette 500 µL of lysis buffer onto the bead slurry. Mix the tube by inverting five times. Precipitate the beads using a magnetic rack.

14.6 Briefly centrifuge the samples for 00:00:05 . Aspirate the buffer. 5s

15 Elute the protein by heating the samples at 95 °C in 2x LDS loading buffer for 00:10:00 . Pipette the eluted sample into a new eppendorf tube or directly in to the gel. 10m

Gel electrophoresis and immunoblotting 2h 10m 5s

16 Prepare samples at desired concentration, add 1 mM DTT and 1x LDS loading buffer.

17 Incubate samples at 95 °C for 00:05:00 .

18 During this incubation, prepare gel apparatus with NuPAGE™ Bis-Tris Mini Protein Gels, 4–12%, 1.0–1.5 mm (ThermoFisher) and 1x MOPS running buffer.

19 Load samples into gel and run until dye front reaches bottom (180 V, roughly 60 min).

20 Remove gel and set up transfer cassette with nitrocellulose membrane.

21 Transfer at 30-35 V for 01:30:00 in 1x transfer buffer.

22 Block membrane with 5% BSA in PBST for 01:00:00 at 22 °C .

23 Incubate membrane with primary antibodies in 5% BSA and 0.03% sodium azid in PBST Overnight at 4 °C .

Note

NOTE: Optimal primary antibody incubation time and temperature can be determined empirically for a given primary antibody

24 Wash membrane with PBST for five minutes. Repeat a total of 3 times.

24.1 Wash membrane for 00:05:00 with PBST (1/3).

24.2 Wash membrane for 00:05:00 with PBST (2/3).

24.3 Wash membrane for 00:05:00 with PBST (3/3).

25 Incubate membrane with secondary antibodies (1:5,000) in 5% milk in PBST for 01:00:00 at 22 °C .

26 Wash membrane with PBST. Repeat a total of 3 times.

26.1 Wash membrane for 00:05:00 with PBST (1/3).

26.2 Wash membrane for 00:05:00 with PBST (2/3).

26.3 Wash membrane for 00:05:00 with PBST (3/3).

27 Image membranes using a BioRad VersaDoc imaging system.

Protocol references

https://www.ptglab.com/products/pictures/pdf/gtma_Manual_GFP-Trap_Magnetic_Agarose.pdf

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