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HA Immunoprecipitation

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We use this protocol and it's working

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Abstract

This protocol details steps taken to perform HA tag immunoprecipitations.

Materials

DMEM (Thermo Fisher Scientific, 11965-092)
FBS (Thermo Fisher Scientific, 16140-071)
Penicillin/Streptomycin (Thermo Fisher Scientific, 15140122)
CellStripper (Corning, 356230)
PBS [1.1 mM KH2PO4 (J.T. Baker, 3246-01), 155.2 mM NaCl (Sigma-Aldrich, 3624-05), and 3 mM Na2HPO4 (J.T. Baker, 3828-05)]
Lysis Buffer [50 mM Tris Base (American Bio, AB02000-05000), 150 mM NaCl (Sigma-Aldrich, 3624-05), 1% Triton X-100 (Sigma-Aldrich, X100), 1 mM EDTA (Sigma-Aldrich, 03690) supplemented with cOmplete mini EDTA-free protease inhibitor (Roche, 11836170001) and PhosSTOP (Roche, 4906837001)
Coomassie Plus Protein Assay Reagent (ThermoFisher Scientific, 23236)
Laemmli Buffer [80 mM Tris-HCl pH 6.8 (American Bio, AB020000-05000 / HCl, Sigma-Aldrich, 3624-06), 25.3% Glycerol (American Bio, AB00751), 2.67% SDS (American Bioanalytical, AB01920-00500), and Bromphenol Blue (Sigma-Aldrich, B5525) supplemented with 6.187% fresh b-mercaptoethanol (Sigma-Aldrich, M3148)]
anti-HA beads (Thermo Fisher Scientific, 88837)
TBST [10 mM Tris Base (American Bio AB02000-05000), 150 mM NaCl (Sigma-Aldrich, 3624-05), 0.1% Tween 20 (Sigma-Aldrich, P7949)]

Day 0

1 Split a ~90% confluent 15-cm dish of RAW 264.7 cells into 2 15-cm dishes.

Day 1

1h 19m

2 Add the indicated compound at the provided concentration and time point in fresh DMEM media containing FBS and P/S.

2.1 Proceed to next step if not treating cells.

3 Wash each dish 2X with cold PBS on ice.

4 Remove the PBS, scrape each well with 700 µL of ice-cold lysis buffer, and transfer the lysates to Eppendorf tubes.

4.1 Lysis Buffer: 50 mM Tris Base, 150 mM NaCl, 1% Triton X-100, and 1 mM EDTA supplemented with cOmplete mini EDTA-free protease inhibitor and PhosSTOP.

5 Incubate on 00:05:00 . 5m

6 Centrifuge 14000 rpm, 4°C, 00:08:00 lysates. 8m

7 Measure the protein concentration of the supernatant using Coomassie Plus Protein Assay Reagent as per the manufacturer's instructions.

8 To generate the Whole Cell Lysate: mix an aliquot of the supernatant/lysate 1:1 with Laemmli Buffer and heat at 95 °C for 00:03:00 . 3m

8.1 Laemmli mBuffer: 80 mM Tris-HCl pH 6.8, 25.4% Glycerol, 2.67% SDS, and Bromophenol Blue supplemented with 6.187% fresh B-mercaptoethanol.

9 Create a mastermix of anti-HA beads (10 µL of beads per sample) and wash 3X with ice-cold lysis buffer.

10 Immunoprecipitate lysates using 10 µL anti-HA beads by incubating rotating end-over-end for 01:00:00 at 4 °C . 1h

10.1 Ensure the same amount of protein was used for each sample and where needed, supplement samples with lysis buffer to ensure the same volume is used.

11 Wash beads 3X with 0.1% TBST.

11.1 TBST: 10 mM Tris Base, 150 mM NaCl, 0.1% Tween 20.

12 Wash beads 1X with mqH2O and transfer to new Eppendorf tubes.

13 Elute samples by incubating beads with 40 µL Laemmli buffer and warming at 95 °C for 00:03:00 . 3m

14 Transfer the eluant to a new Eppendorf tube and supplement with 6.187% B-mercaptoethanol.

15 Subject samples to electrophoresis and immunoblotting as in Protein Lysate and Immunoblotting.