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IP-mass spectrometry

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We use this protocol and it's working

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Abstract

IP-mass spectrometry for protein of interset

Guidelines

PDAC cells expressing Flag-SMARCA5 in the doxycycline-inducible manner were collected after Dox was added 48h. Cells were resuspended in hypotonic buffer A and homogenized. Nuclei were collected by centrifugation. Then, the nuclei were resuspended in two packed cell pellet volumes of buffer C (25% Glycerol, 600mM KCl, 0.2mM EDTA pH = 8.0, 20mM HEPES pH = 7.9, 1.5mM Mgcl2, 0.5mM DTT, 0.5mM PMSF, protease inhibitor cocktail), rocked in 4C for 1h, centrifuged (15 min, 21000g, 4C) and the nuclear extract (NE) was collected. NE was centrifuged and the supernatant was then pre-cleared with Protein A/G Sepharose beads before proceeding with immunoprecipitation (IP). M2 beads (Sigma, A2220) were equilibrated with wash buffer and then incubated with cell nuclear extracts at 4°C for at least 4 h. The beads were washed with wash buffer (20 mM HEPES pH 7.9, 0.2 mM EDTA, 150 mM NaCl, 10% glycerol, 0.2% Triton X-100, 0.5 mM DTT and 0.5mM PMSF) and bound proteins were eluded with 3X FLAG peptide (GL Biochem, 56305) and run on SDS–PAGE for Mass spectrometry at the Protein Chemistry Facility at the Center for Biomedical Analysis of Tsinghua University.

Materials

- PDAC cells expressing Flag-SMARCA5 (doxycycline-inducible)
- Doxycycline (Dox)
- Hypotonic buffer A
- Buffer C components: 25% Glycerol; 600mM KCl; 0.2mM EDTA pH = 8.0; 20mM HEPES pH = 7.9; 1.5mM Mgcl2; 0.5mM DTT; 0.5mM PMSF; protease inhibitor cocktail
- Protein A/G Sepharose beads
- M2 beads (Sigma, A2220)
- Wash buffer components: 20 mM HEPES pH 7.9; 0.2 mM EDTA; 150 mM NaCl; 10% glycerol; 0.2% Triton X-100; 0.5 mM DTT; 0.5 mM PMSF
- 3X FLAG peptide (GL Biochem, 56305)
- SDS–PAGE reagents and equipment
- Centrifuge capable of 21,000g
- Rocker/incubator capable of 4C
- Protease inhibitor cocktail
- Mass spectrometry services (Protein Chemistry Facility, Center for Biomedical Analysis of Tsinghua University)

Before start

Induce PDAC cells with doxycycline (Dox) and collect cells 48 h after Dox addition.

1 Resuspend cell pellets in hypotonic buffer A (10mM KCl, 10mM HEPES pH=7.9, 1.5mM $MgCl_2$, 0.5mM DTT, 0.5mM PMSF, protease inhibitor cocktail) and homogenize to lyse the cells.

2 Collect nuclei by centrifugation.

3 Resuspend nuclei in two packed cell pellet volumes of buffer C (25% Glycerol; 600 mM KCl; 0.2 mM EDTA pH = 8.0; 20 mM HEPES pH = 7.9; 1.5 mM MgCl2; 0.5 mM DTT; 0.5 mM PMSF; protease inhibitor cocktail), then rock the suspension at 4°C for 1 h.

4 Centrifuge the suspension for 15 min at 21,000 g and 4°C and collect the nuclear extract (NE).

5 Centrifuge the NE, collect the supernatant, and pre-clear the supernatant with Protein A/G Sepharose beads prior to immunoprecipitation.

6 Equilibrate M2 beads (Sigma, A2220) with wash buffer, then incubate the beads with the pre-cleared cell nuclear extracts at 4°C for at least 4 h.

7 Wash the beads with wash buffer (20 mM HEPES pH 7.9; 0.2 mM EDTA; 150 mM NaCl; 10% glycerol; 0.2% Triton X-100; 0.5 mM DTT; 0.5 mM PMSF) to remove non-specifically bound proteins.

8 Elute bound proteins from the beads using 3X FLAG peptide (GL Biochem, 56305).

9 Separate the eluted proteins by SDS–PAGE and submit the gel bands/samples for mass spectrometry analysis at the Protein Chemistry Facility, Center for Biomedical Analysis of Tsinghua University.

Acknowledgements

Mass spectrometry was performed at the Protein Chemistry Facility at the Center for Biomedical Analysis of Tsinghua University.