Immunoprecipitation of ROR1

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[Abstract] ROR1 is a receptor tyrosine kinase family member studied for its roles in development and cancer. Here we describe a protocol for immunoprecipitation of endogenous ROR1 from t(1;19) (a disease subtype categorized by its chromosome translocation) acute lymphoblastic leukemia immortalized cell lines.

Materials and Reagents

1. ROR1-positive cells (*e.g.* RCH-ACV cells, Kasumi-2 cells, 697 cells)
2. Cell lysis buffer (Cell Signaling Technology, catalog number: 9803)
3. Protease inhibitor cocktail (cOmplete Mini EDTA-free) (Roche, catalog number: 04693159001)
4. Phosphatase inhibitor cocktail II (Sigma-Aldrich, catalog number: P5726)
5. Antibody specific for ROR1 (R&D Systems, catalog number: AF2000)
6. Isotype matched control (R&D Systems, catalog number: AB-108-C)
7. Protein G Agarose beads (EMD Millipore, catalog number: 16-266)
8. SDS
9. BSA
10. DTT

Equipment

1. P1000 pipette
2. Centrifuge

Procedure

1. 10^7 cells (such as RCH-ACV, Kasumi-2, 697 cells) are pelleted, washed 1x in PBS, pelleted, and thoroughly resuspended in 500 µl ice-cold lyses buffer supplemented with

protease inhibitor cocktail and phosphatase inhibitor cocktail II (according to manufacturers' instruction) using a P1000 pipette.

2. The lysis reaction is kept on ice and vortexed at max speed 3 times for 3-second bursts. The reaction is kept on ice for 5 min between each vortexing.
3. Following lysis, samples are centrifuged at max-speed for 15 min at 4 °C.
4. Following pelleting, the supernatant is transferred to a fresh tube, kept on ice, and subjected to Bradford protein concentration analysis. Lysate is diluted to 2 mg/ml in lysis buffer for IP.
5. 3 µg of antibody specific for ROR1 or an isotype matched control was incubated with cell extracts (500 µl at 2 mg/ml) by rotating overnight.
6. Protein G Agarose beads were prepared for precipitation by washing twice in lysis buffer and blocking for 1 h (nutating) at 4 °C in lysis buffer containing 0.5% BSA.
7. Following blocking, beads were pelleted and resuspended in lysis buffer to a 50% slurry, and 40 µl 50% slurry was added to lysate and rocked at 4 °C for 1 h.
8. Following precipitation, beads were washed in 500 µl lysis buffer 3x by nutating at 4 °C for 20 min.
9. Proteins were eluted from the beads by boiling in an aqueous solution with 2% SDS and 25 mM DTT.

Acknowledgments

This protocol was adapted from Bicocca *et al.* (2012).

References

1. Bicocca, V. T., Chang, B. H., Masouleh, B. K., Muschen, M., Loriaux, M. M., Druker, B. J. and Tyner, J. W. (2012). Crosstalk between ROR1 and the Pre-B cell receptor promotes survival of t(1;19) acute lymphoblastic leukemia. *Cancer Cell* 22(5): 656-667.

