ROR1 Flow Cytometry

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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 analysis of ROR1 surface expression in acute lymphoblastic leukemia immortalized cell lines by flow cytometry.

Materials and Reagents

1. Cells (*e.g.* RCH-ACV cells, Kasumi-2 cells, REH cells, MHH-CALL-2 cells)
2. FBS
3. Antibody specific for ROR1 (R&D Systems, catalog number: AF2000)
4. Goat IgG (R&D Systems, catalog number: AB-108-C)
5. Donkey Anti-goat IgG-Phycoerythrin (R&D Systems, catalog number: F0107)

Equipment

1. Centrifuge
2. FACSAria (BD Biosciences)

Procedure

1. Actively cultured RCH-ACV, Kasumi-2, REH, and MHH-CALL-2 cells were pelleted and washed once in PBS and then resuspended in PBS wash buffer containing 2% FBS (1 million cells in 50 µl of buffer).
2. 1 x 10^6 cells were immunostained at room temperature for 30 min with 1 µg of antibody specific for ROR1 or Goat IgG (do not need to rotate the reaction).
3. Cells were washed 3 times with 500 µl PBS wash buffer.
4. Cells were stained with Donkey Anti-goat IgG-Phycoerythrin (10 µl Donkey Anti-goat IgG-Phycoerythrin is diluted into 90 µl PBS wash buffer). Incubate at room temperature in the dark for 15 min.

5. Samples are washed 1x with 500 µl PBS wash buffer and then resuspended in 200 µl PBS wash buffer for analysis.
6. Samples were analyzed on a BD FACSAria.

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.

