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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. FACS Aria (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 μ l of buffer).
2. 1×10^6 cells were immunostained at room temperature for 30 min with 1 μ g of antibody specific for ROR1 or Goat IgG (do not need to rotate the reaction).
3. Cells were washed 3 times with 500 μ l PBS wash buffer.

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4. Cells were stained with Donkey Anti-goat IgG-Phycoerythrin (10 μ l Donkey Anti-goat IgG-Phycoerythrin is diluted into 90 μ l PBS wash buffer). Incubate at room temperature in the dark for 15 min.
5. Samples are washed 1x with 500 μ l PBS wash buffer and then resuspended in 200 μ l PBS wash buffer for analysis.
6. Samples were analyzed on a BD FACS Aria.

Acknowledgments

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

References

1. Bicocca VT, Chang BH, Masouleh BK, Muschen M, Loriaux MM, Druker BJ, Tyner JW. Crosstalk between ROR1 and the Pre-B cell receptor promotes survival of t(1;19) acute lymphoblastic leukemia. *Cancer Cell*. 2012; 22(5):656–667. [PubMed: 23153538]