

## 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  $\mu$ l of buffer).
2.  $1 \times 10^6$  cells were immunostained at room temperature for 30 min with 1  $\mu$ g of antibody specific for ROR1 or Goat IgG (do not need to rotate the reaction).
3. Cells were washed 3 times with 500  $\mu$ l PBS wash buffer.
4. Cells were stained with Donkey Anti-goat IgG-Phycoerythrin (10  $\mu$ l Donkey Anti-goat IgG-Phycoerythrin is diluted into 90  $\mu$ l PBS wash buffer). Incubate at room temperature in the dark for 15 min.

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5. Samples are washed 1x with 500  $\mu$ l PBS wash buffer and then resuspended in 200  $\mu$ l 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.