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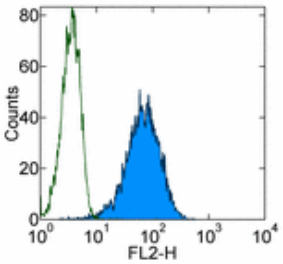
Anti-Mouse CD34 Purified

Catalog Number: 14-0341
RUO: For Research Use Only



Staining of NIH/3T3 cell line with 1.0 µg of Rat IgG2a Isotype Control Purified (cat. 14-4321) (open histogram) or 1.0 µg of Anti-Mouse CD34 Purified (filled histogram) followed by Anti-Rat IgG Boitin (cat. 13-4813) and Streptavidin PE (cat. 12-4317). Total viable cells were used for analysis.

Product Information

Contents: Anti-Mouse CD34 Purified
REF Catalog Number: 14-0341
Clone: RAM34
Concentration: 0.5 mg/ml
Host/Isotype: Rat IgG2a, κ

Formulation: aqueous buffer, 0.09% sodium azide, may contain carrier protein/stabilizer
Temperature Limitation: Store at 2-8°C.
LOT Batch Code: Refer to Vial
Use By: Refer to Vial
Caution, contains Azide

Description

The RAM34 monoclonal antibody reacts with mouse CD34, also known as mucosialin. It has been reported that the RAM34 antibody can be used to detect $CD34^+Sca\text{-}1^+c\text{-}Kit^+$ cells. Upon further optimization, we have determined that the most effective RAM34 formats for this combination of antigen detection are biotin, FITC, and Alexa Fluor 647. Preliminary data suggest that some antibody conjugates (such as PE and APC fluorochromes) may negatively impact detection of CD34. Interestingly, the PE RAM34 conjugate is suitable for the staining of epithelial cells (NIH3T3).

CD34, a highly glycosylated (approximately 90-120 kDa) member of the sialomucin family, is expressed by capillary endothelial cells, BM stoma and a small subpopulation of mouse bone marrow cells. RAM34 has been used to purify mouse HSCs to near homogeneity. Unlike in humans, primitive adult mouse bone marrow HSCs were detected in the CD34 low to negative fraction. CD34 expressed on endothelial cells is a ligand for CD62L and plays a role in adhesion. Simultaneous staining of mouse bone marrow cells with a cocktail of antibodies to lineage markers (CD3, CD11b, Ly6G, TER-119 and CD45R/B220) reveals a subset of total BM cells staining with RAM34 that can express undetectable to low levels of the mentioned lineage markers.

Note: When staining with the RAM34 antibody an incubation time of 90 minutes is recommended to obtain the optimal signal to noise signal.

Applications Reported

This RAM34 antibody has been reported for use in flow cytometric analysis, immunohistology staining of frozen tissue sections (acetone-fixed only, not formalin-fixed), and immunohistology staining of paraffin embedded tissue sections.

Applications Tested

The RAM34 antibody is routinely tested by flow cytometric analysis of a mouse cell line (NIH/3T3) and mouse bone marrow (BM) cell suspensions. When using conjugates of RAM34 for staining of mouse BM cells, we routinely perform a two-color analysis using RAM34 in combination with Lineage Cocktail (cat. 88-7774) to identify lineage-committed BM cells and better visualize the minor subset of Lineage negative/low cells that normally stain with RAM34 as reported in the literature. Gating strategies that exclude cells with low level expression of lineage markers may significantly decrease the total number of RAM34-positive cells. If using a Lineage Cocktail and/or other markers such as CD117/c-Kit or Ly6AE/Sca-1, it is best to analyze data on two-color plots (dot-plots or contour-plots, etc.) for best visualization of the staining. If you are using only one-color staining, analyze data as a two-parameter plot of RAM34 staining vs. Forward Light Scatter (FSC). Collecting and analyzing >10,000 total events per sample is helpful in increasing the number of RAM34-positive cells.

It is recommended to use 1 µg per million BM cells in a 100 µl total staining volume. It is recommended that the antibody be carefully titrated for optimal performance in the assay of interest. For more detailed information on staining with RAM34, please reference the following publication PubMed.

References

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Singh P, Yao Y, Weliver A, Broxmeyer HE, Hong SC, Chang CH. Vaccinia virus infection modulates the hematopoietic cell compartments in the bone marrow. Stem Cells. 2008 Apr;26(4):1009-16. (RAM34, FC, PubMed)

Lorenz K, Grashoff C, Torka R, Sakai T, Langbein L, Bloch W, Aumailley M, Fässler R. Integrin-linked kinase is required for epidermal and hair follicle morphogenesis. J Cell Biol. 2007 May 7;177(3):501-13 (RAM34, IHC, PubMed)

Iida M, Ihara S, Matsuzaki T. Hair cycle-dependent changes of alkaline phosphatase activity in the mesenchyme and epithelium in mouse vibrissal follicles. Dev Growth Differ. 2007 Apr;49(3):185-95. (RAM34, IHC frozen, PubMed)

Park TJ, Boyd K, Curran T. Cardiovascular and craniofacial defects in Crk-null mice. Mol Cell Biol. 2006 Aug;26(16):6272-82. (RAM34, IHC paraffin)

Osawa M, Hanada K, Hamada H, Nakauchi H. Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. Science. 1996 Jul 12;273(5272):242-5.

Related Products
11-4317 Streptavidin FITC
11-4811 Anti-Rat IgG FITC
12-4317 Streptavidin PE
13-4813 Anti-Rat IgG Biotin (Polyclonal)
14-4321 Rat IgG2a K Isotype Control Purified
17-4317 Streptavidin APC

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