

Anti-Erythroid Cells antibodies, rat

For research use only

9 µg equal 60 tests, 30 µg equal 200 tests. One test corresponds to labeling of 10⁶ cells.

Product	Content	Order no.
Anti-Erythroid Cells-VioBright FITC	9 µg in 300 µL	130-109-623
Anti-Erythroid Cells-VioBright FITC	30 µg in 1 mL	130-109-564
Anti-Erythroid Cells-PE	9 µg in 300 µL	130-109-619
Anti-Erythroid Cells-PE	30 µg in 1 mL	130-109-560
Anti-Erythroid Cells-APC	9 µg in 300 µL	130-109-620
Anti-Erythroid Cells-APC	30 µg in 1 mL	130-109-561
Anti-Erythroid Cells-PE-Vio770	9 µg in 300 µL	130-109-621
Anti-Erythroid Cells-PE-Vio770	30 µg in 1 mL	130-109-562
Anti-Erythroid Cells-APC-Vio770	9 µg in 300 µL	130-109-622
Anti-Erythroid Cells-APC-Vio770	30 µg in 1 mL	130-109-563
Anti-Erythroid Cells-Biotin	9 µg in 300 µL	130-109-618
Anti-Erythroid Cells-Biotin	30 µg in 1 mL	130-109-559

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

Technical data and background information

Antigen	Erythroid Cells
Clone	REA629
Isotype	recombinant human IgG1
Isotype control	REA Control antibodies
Alternative names of antigen	Ly-76, Ly76, MRC, OX-83, TER-119, Ter119
Distribution of antigen	red blood cells
Product format	Reagents are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	Cells should be stained prior to fixation, if formaldehyde is used as a fixative.
Storage	Store protected from light at 2–8 °C. Do not freeze.

Clone REA629 recognizes a 52 kDa glycoprotein A-associated protein, also known as Ly-76. It is expressed on rat erythrocytes and erythroid progenitors, but not on granulocytes, monocytes, or lymphocytes.

Additional information: Clone REA629 displays negligible binding to Fc receptors.

Reagent requirements

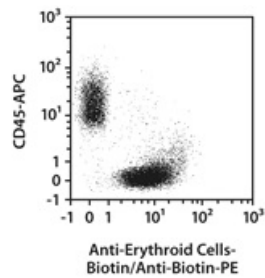
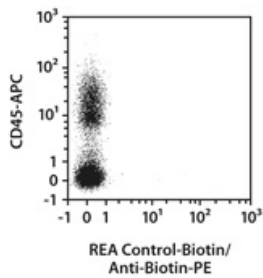
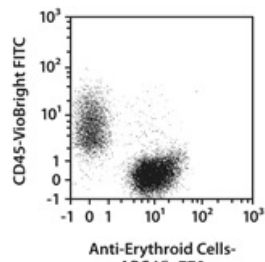
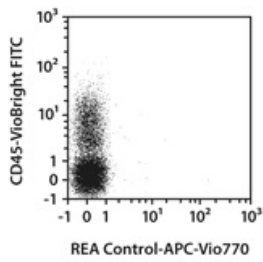
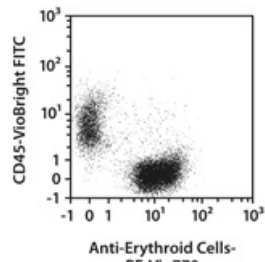
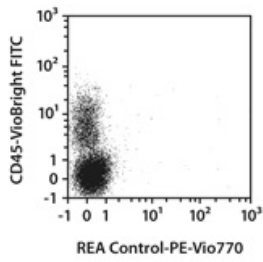
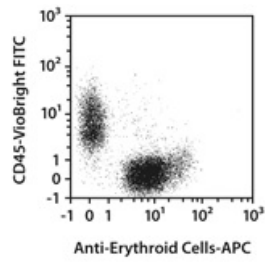
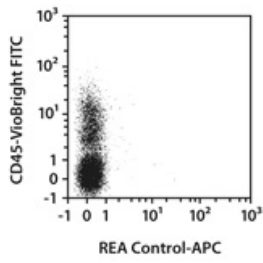
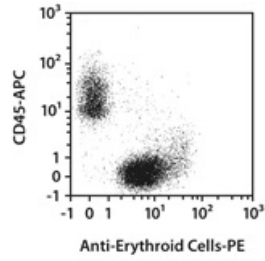
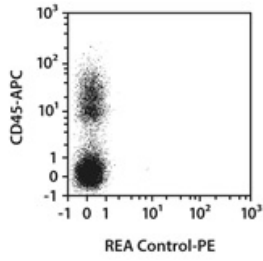
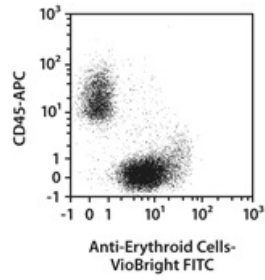
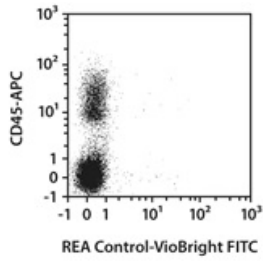
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS[®] BSA Stock Solution (# 130-091-376) 1:20 with autoMACS[®] Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).
Note: EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) Fluorochrome-conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with biotinylated antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) for flow cytometric exclusion of dead cells without fixation.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

Protocol for cell surface staining

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:10 for up to 10⁶ cells/50 µL of buffer.
 - Volumes given below are for up to 10⁶ nucleated cells. When working with fewer than 10⁶ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10⁶ nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).
1. Determine cell number.
 2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
 3. Resuspend up to 10⁶ nucleated cells per 45 µL of buffer.
 4. Add 5 µL of the antibody.
 5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
Note: Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
 6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
 7. (Optional) If biotinylated antibody was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of fluorochrome-conjugated anti-biotin antibody, and continue as described in steps 5 and 6.
 8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

Examples of immunofluorescent staining

Bone marrow cells from Wistar rats were stained with Anti-Erythroid Cells antibodies or with the corresponding REA Control antibodies (left images) as well as with CD45 antibodies. Flow cytometry was performed using the MACSQuant[®] Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandem conjugates.



References

1. **Hermans, M. H. et al.** (1991) *In situ* visualization of hemopoietic cell subsets and stromal elements in rat and mouse bone marrow by immunostaining of frozen sections. *J. Histochem. Cytochem.* 39(12): 1627–1634.
2. **Kimoto, T. et al.** (2013) Interlaboratory trial of the rat Pig-a mutation assay using an erythroid marker HIS49 antibody. *Mutat. Res.* 755(2): 126–134.
3. **Zheng, Y. W. et al.** (2014) The CD133⁺CD44⁺ precancerous subpopulation of oval cells is a therapeutic target for hepatocellular carcinoma. *Stem Cells Dev.* 23(18): 2237–2249.

Warranty

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