CD352 (NTB-A) antibodies, mouse

For research use only

9 µg equal 60 tests, 30 µg equal 200 tests. One test corresponds to labeling of 10^6 cells.

Product	Content	Order no.
CD352 (NTB-A)-FITC	9 µg in 300 µL	130-109-901
CD352 (NTB-A)-FITC	30 µg in 1 mL	130-109-859
CD352 (NTB-A)-PE	9 µg in 300 µL	130-109-902
CD352 (NTB-A)-PE	30 µg in 1 mL	130-109-860
CD352 (NTB-A)-APC	9 µg in 300 µL	130-109-903
CD352 (NTB-A)-APC	30 µg in 1 mL	130-109-861
CD352 (NTB-A)-PE-Vio770	9 µg in 300 µL	130-109-904
CD352 (NTB-A)-PE-Vio770	30 µg in 1 mL	130-109-862
CD352 (NTB-A)-APC-Vio770	9 µg in 300 µL	130-109-905
CD352 (NTB-A)-APC-Vio770	30 µg in 1 mL	130-109-863
CD352 (NTB-A)-Biotin	9 µg in 300 µL	130-109-900
CD352 (NTB-A)-Biotin	30 µg in 1 mL	130-109-858

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	CD352 (NTB-A)
Clone	13G3
Isotype	mouse IgG2aκ
Isotype control	Mouse IgG2a – isotype control antibodies
Alternative names of antigen	SLAM Family Receptor, SLAMF6, SLAM F6, LY108, Lymphocyte antigen 108, SLAM family member 6
Molecular mass of antigen [kDa]	36
Distribution of antigen	NK cells, B cells, T 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 13G3 recognizes the mouse CD352 antigen, a single-pass type I membrane protein, also known

as SLAM family member 6 (SLAM6) or NK, T, and B cell antigen (NTB-A) belonging to the CD2 subfamily of the immunoglobulin superfamily. It is expressed on NK, T, and B cells. It undergoes tyrosine phosphorylation and associates with the Src homology 2 domain-containing protein (SH2D1A) as well as with SH2 domain-containing phosphatases. CD352 plays a role in NK cell cytotoxicity and T cell cytokine responses. It controls neutrophil functions and serves as a regulator of both innate and adaptive immune responses.

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 Ca^{2+} or Mg^{2+} are not recommended for use.
- (Optional) FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor–mediated antibody labeling.
- (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.

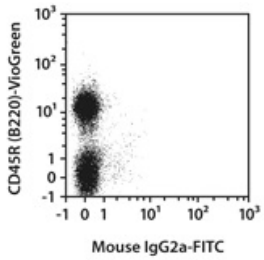
Protocol for cell surface staining

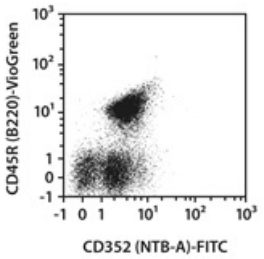
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:10 for up to 10^6 cells/50 µL of buffer.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^6 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

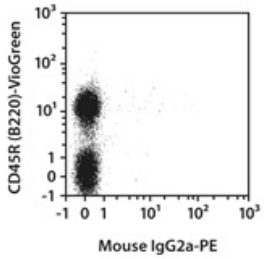
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 45 µL of buffer.
4. Add 5 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher 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.

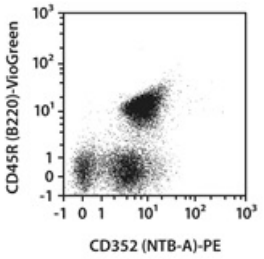
Examples of immunofluorescent staining

Splenocytes from C57BL/6 mice were stained with CD352 (NTB-A) antibodies or with the corresponding isotype control antibodies (left image) as well as with CD45R (B220) 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.


CD45R (B220)-VioGreen
Mouse IgG2a-FITC


CD45R (B220)-VioGreen
CD352 (NTB-A)-FITC


CD45R (B220)-VioGreen
Mouse IgG2a-PE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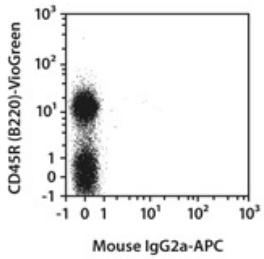
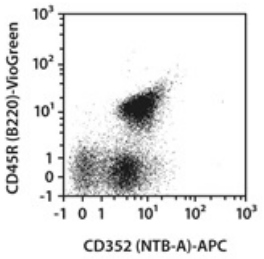
CD352 (NTB-A)-PE


CD45R (B220)-VioGreen
Mouse IgG2a-APC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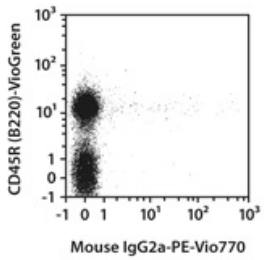
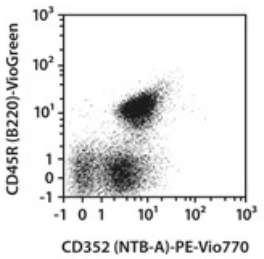
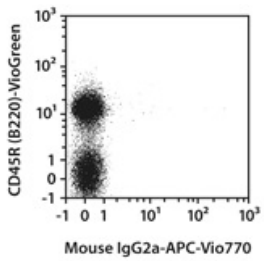
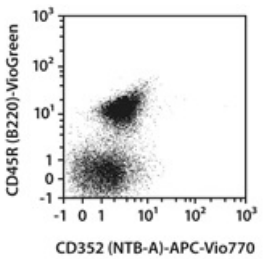
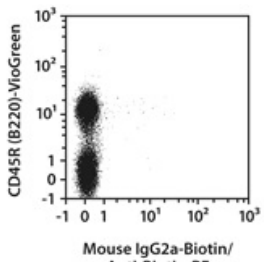
CD352 (NTB-A)-APC

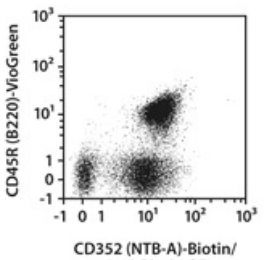

CD45R (B220)-VioGreen
Mouse IgG2a-PE-Vio770


CD45R (B220)-VioGreen
CD352 (NTB-A)-PE-Vio770


CD45R (B220)-VioGreen
Mouse IgG2a-APC-Vio770


CD45R (B220)-VioGreen
CD352 (NTB-A)-APC-Vio770


CD45R (B220)-VioGreen
Mouse IgG2a-Biotin/
Anti-Biotin-PE


CD45R (B220)-VioGreen
CD352 (NTB-A)-Biotin/
Anti-Biotin-PE

References

1. **Peck, S. R. *et al.*** (2000) Ly108: a new member of the mouse CD2 family of cell surface proteins. Immunogenetics 52(1-2): 63–72.
2. **Howie, D. *et al.*** (2005) Cutting edge: the SLAM family receptor Ly108 controls T cell and neutrophil functions. J. Immunol. 174(10): 5931–5935.
3. **Griewank, K. *et al.*** (2007) Homotypic interactions mediated by Slamf1 and Slamf6 receptors control NKT cell lineage development. Immunity 27(5): 751–762.

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