CD335 (NKp46) antibodies, human

For research use only

6
One test corresponds to labeling of up to 10 cells in a total volume of 100 µL

Product	Content	Order no.
CD335 (NKp46)-Biotin	for 100 tests	130-112-118
CD335 (NKp46)-PE	for 30 tests	130-112-279
CD335 (NKp46)-PE	for 100 tests	130-112-121
CD335 (NKp46)-APC	for 30 tests	130-112-280
CD335 (NKp46)-APC	for 100 tests	130-112-122
CD335 (NKp46)-PE-Vio615	for 30 tests	130-112-277
CD335 (NKp46)-PE-Vio615	for 100 tests	130-112-119
CD335 (NKp46)-PE-Vio770	for 30 tests	130-112-281
CD335 (NKp46)-PE-Vio770	for 100 tests	130-112-123
CD335 (NKp46)-VioBright 515	for 30 tests	130-112-278
CD335 (NKp46)-VioBright 515	for 100 tests	130-112-120
CD335 (NKp46)-Biotin	for 30 tests	130-112-276

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	CD335 (NKp46)
Clone	REA808
Isotype	recombinant human IgG1
Isotype control	REA Control (S) antibodies
Alternative names of antigen	NK-p46, hNKp46, NCR1, LY94
Entrez Gene ID	9437
Molecular mass of antigen [kDa]	32
Distribution of antigen	NK 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 REA808 recognizes the CD335 antigen, a type I membrane glycoprotein also known as NKp46. CD335 is a member of the natural cytotoxicity receptor (NCR) family which trigger cytotoxicity in NK cells and mediates cell activation. CD335 is directly involved in target cell recognition and lysis and is exclusively expressed on $CD3^-CD56^+$ NK cells, suggesting it to be a universal marker for NK cells.

Additional information: Clone REA808 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 Ca^{2+} or Mg^{2+} 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.

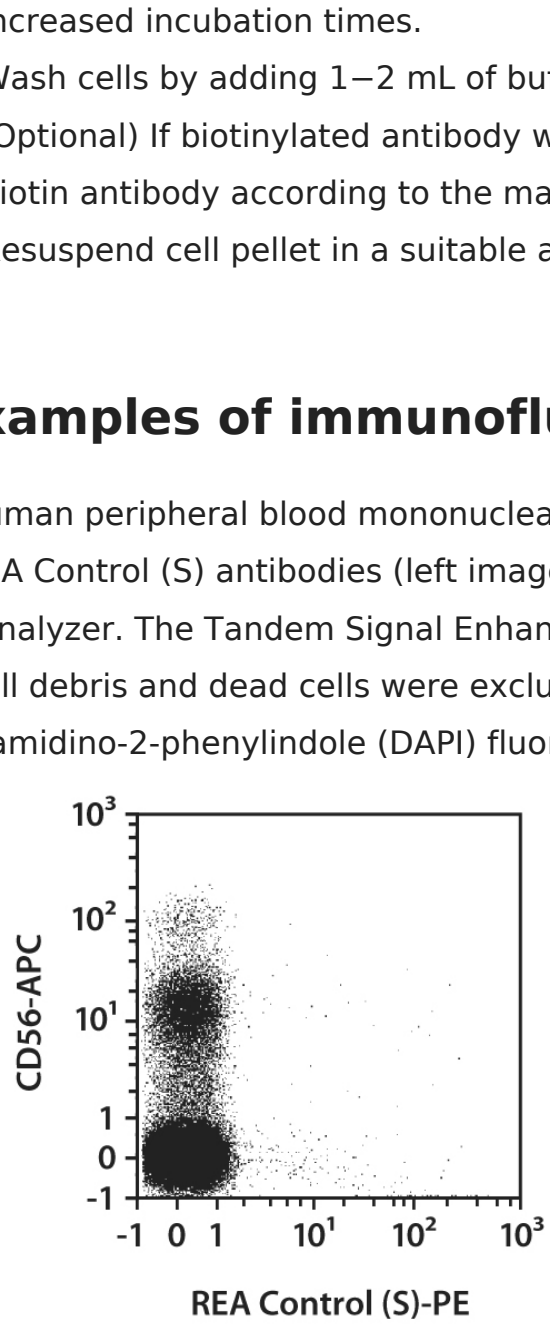
Protocol for cell surface staining

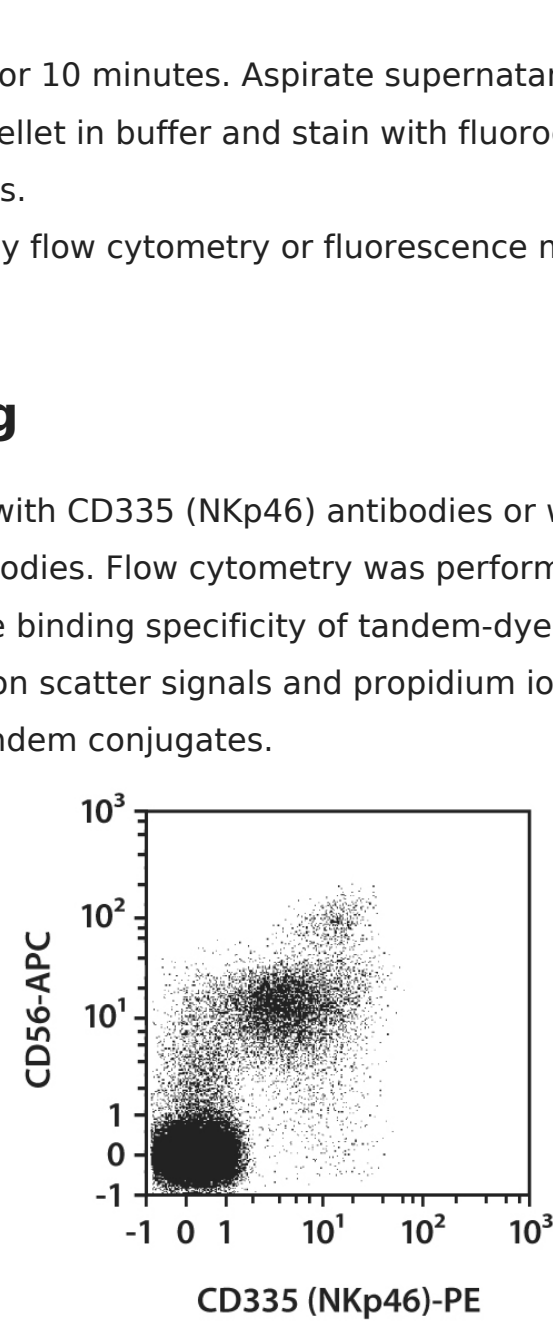
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10^6 cells/100 µL.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly.

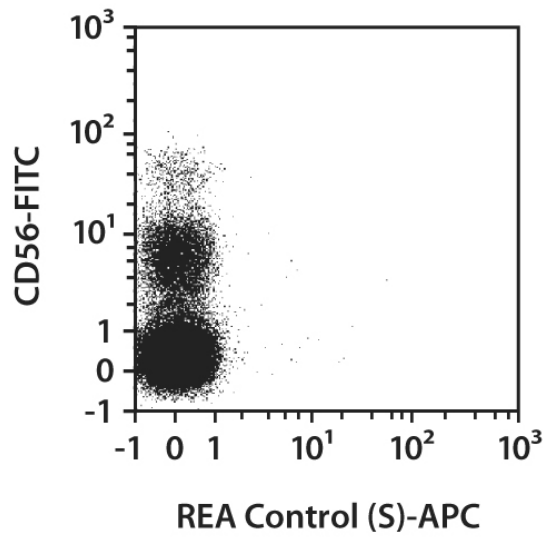
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 98 µL of buffer.
4. Add 2 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher 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 buffer and stain with fluorochrome-conjugated anti-biotin antibody according to the manufacturer's recommendations.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

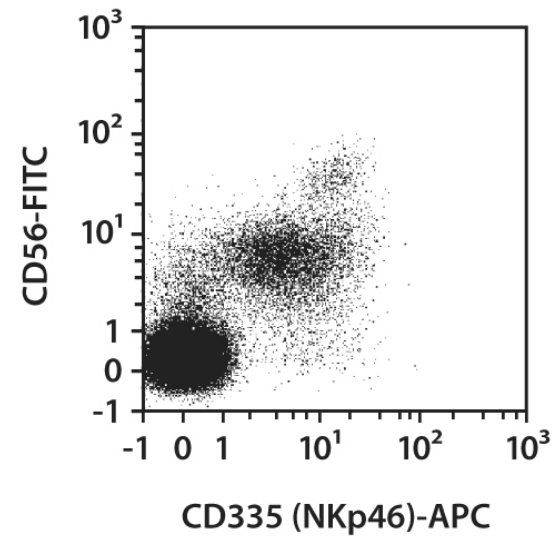
Examples of immunofluorescent staining

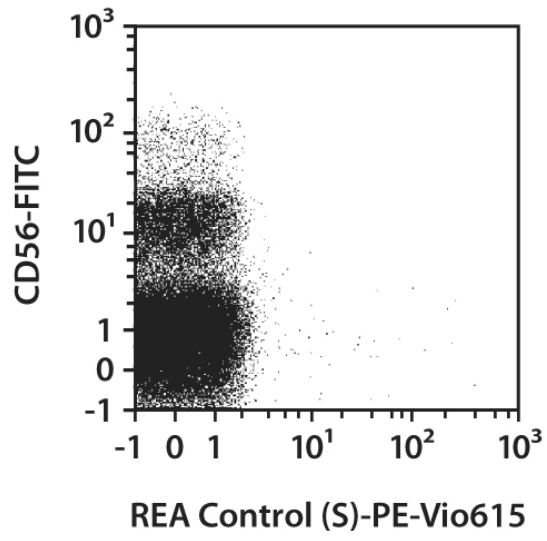
Human peripheral blood mononuclear cells (PBMCs) were stained with CD335 (NKp46) antibodies or with the corresponding REA Control (S) antibodies (left images) as well as with CD56 antibodies. Flow cytometry was performed using the MACSQuant® Analyzer. The Tandem Signal Enhancer has been used to increase binding specificity of tandem-dye–conjugated antibodies. 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.

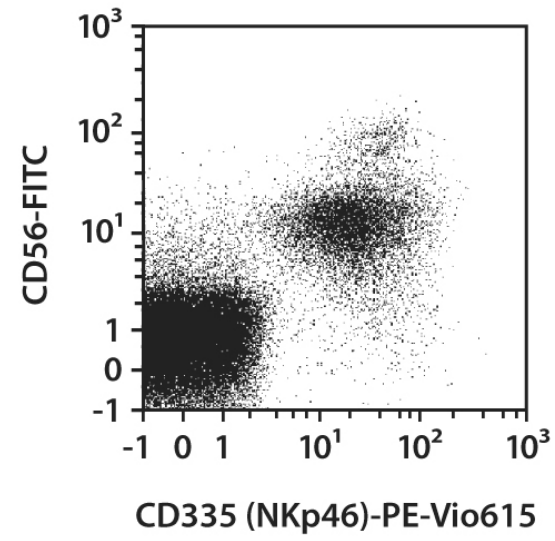


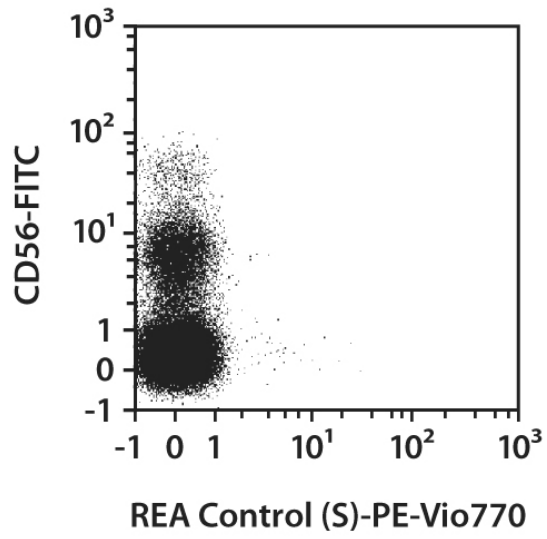


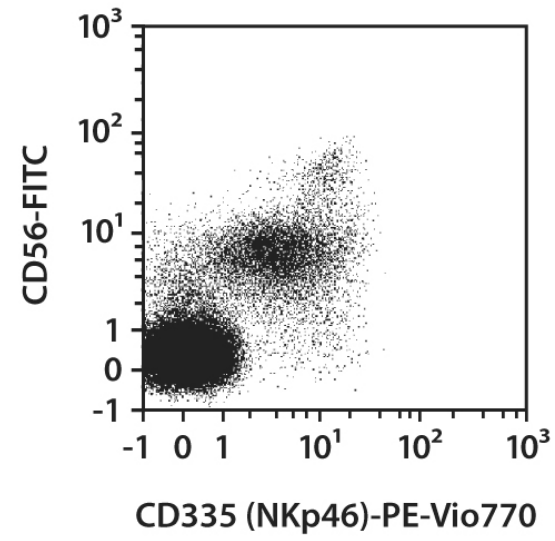


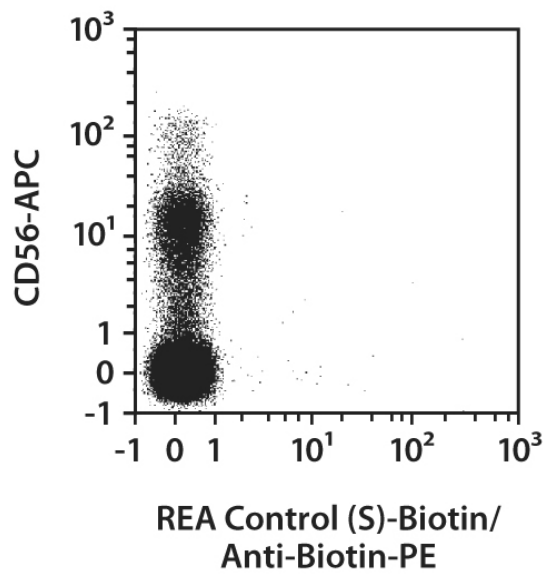


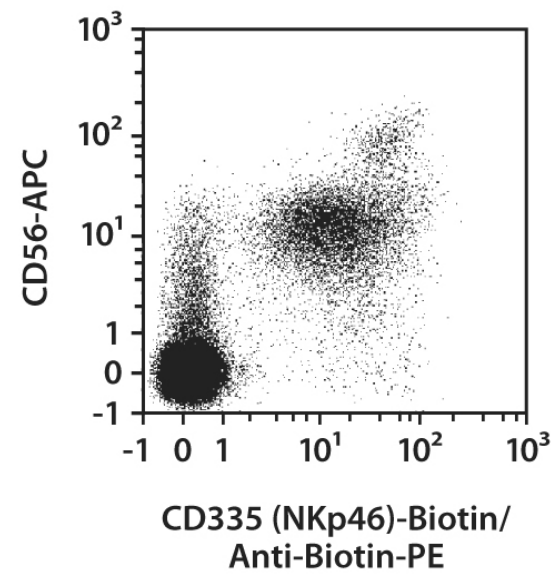












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