# Anti-IgD antibodies, mouse

## For research use only



30 µg equal 100 tests, 150 µg equal 500 tests. One test corresponds to labeling of 10  cells.

| Product | Content | Order no. |
|---|---|---|
| Anti-IgD-Biotin | 150 µg in 1 mL | 130-111-308 |
| Anti-IgD-FITC | 30 µg in 200 µL | 130-111-495 |
| Anti-IgD-FITC | 150 µg in 1 mL | 130-111-309 |
| Anti-IgD-PE | 30 µg in 200 µL | 130-111-496 |
| Anti-IgD-PE | 150 µg in 1 mL | 130-111-310 |
| Anti-IgD-APC | 30 µg in 200 µL | 130-111-497 |
| Anti-IgD-APC | 150 µg in 1 mL | 130-111-311 |
| Anti-IgD-VioBlue | 30 µg in 200 µL | 130-111-501 |
| Anti-IgD-VioBlue | 150 µg in 1 mL | 130-111-315 |
| Anti-IgD-VioGreen | 30 µg in 200 µL | 130-111-502 |
| Anti-IgD-VioGreen | 150 µg in 1 mL | 130-111-316 |
| Anti-IgD-PE-Vio770 | 30 µg in 200 µL | 130-111-498 |
| Anti-IgD-PE-Vio770 | 150 µg in 1 mL | 130-111-312 |
| Anti-IgD-APC-Vio770 | 30 µg in 200 µL | 130-111-499 |
| Anti-IgD-PerCP-Vio700 | 30 µg in 200 µL | 130-111-500 |
| Anti-IgD-PerCP-Vio700 | 150 µg in 1 mL | 130-111-314 |
| Anti-IgD-Biotin | 30 µg in 200 µL | 130-111-494 |

## 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** | IgD |
| **Clone** | REA772 |
| **Isotype** | recombinant human IgG1 |
| **Isotype control** | REA Control antibodies |
| **Alternative names of antigen** | IGHD, Igh-5 |
| **Entrez Gene ID** | 380797 |
| **Molecular mass of antigen [kDa]** | 28 |
| **Distribution of antigen** | B cells |

| | |
|---|---|
| **Product format** | Reagents are supplied in buffer containing stabilizer and 0.05% sodium azide. |
| **Fixation** | The antibody is suited for staining of formaldehyde-fixed cells. |
| **Storage** | Store protected from light at 2–8 °C. Do not freeze. |

Clone REA772 specifically recognizes the mouse immunoglobulin D (IgD) of all tested mouse haplotypes and it does not react with other immunoglobulin isotypes. IgD is expressed by peripheral mature B cells. The Anti-IgD antibody neither activates B cells nor induces proliferation of B cells*in vitro*. Additional information: Clone REA772 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.

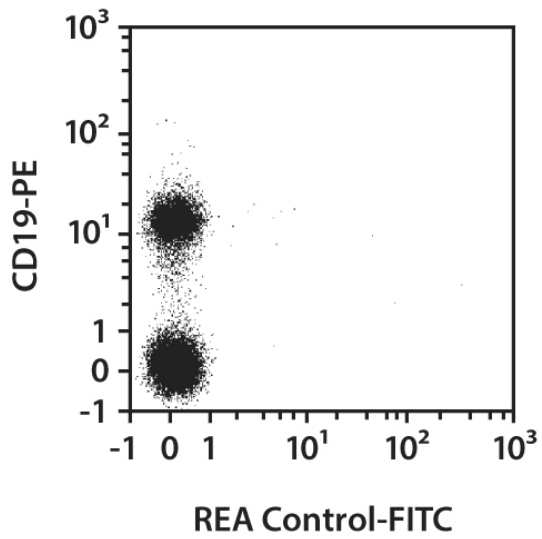
## Protocol for cell surface staining

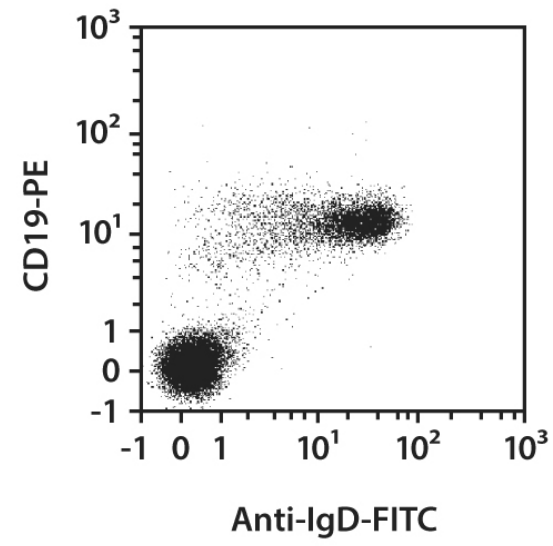
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to $10^6$ cells/100 µL.
- Volumes given below are for up to $10^6$ nucleated cells. When working with fewer than $10^6$ cells, use the same volumes as indicated. When working with higher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

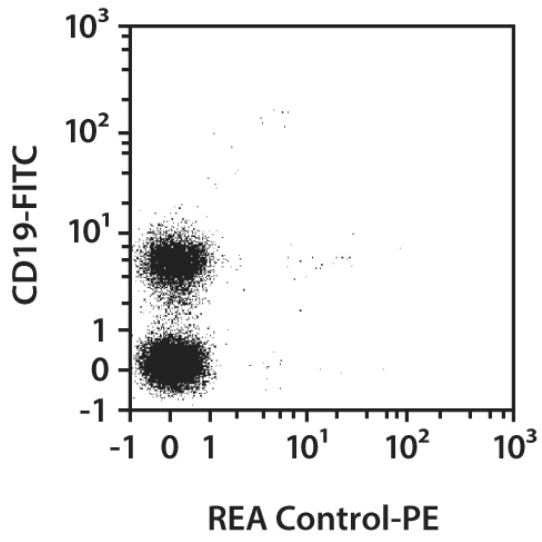
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to $10^6$ nucleated cells per 98 µL of buffer.
4. Add 2 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher 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

Splenocytes from C57BL/6 mice were stained with Anti-IgD antibodies or with the corresponding REA Control antibodies (left image) as well as with CD19 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.


CD19-PE
REA Control-FITC


CD19-PE
Anti-IgD-FITC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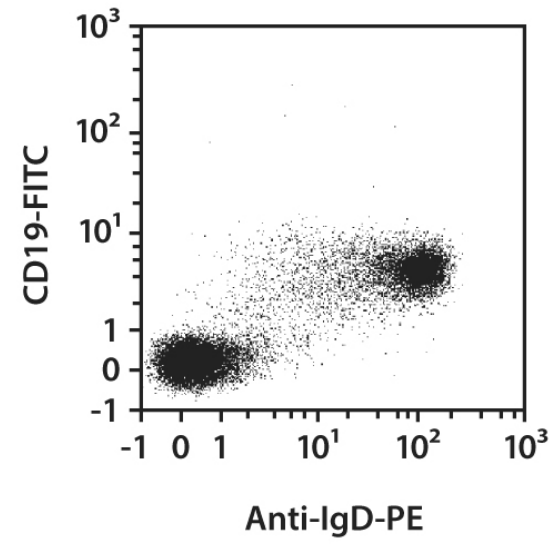
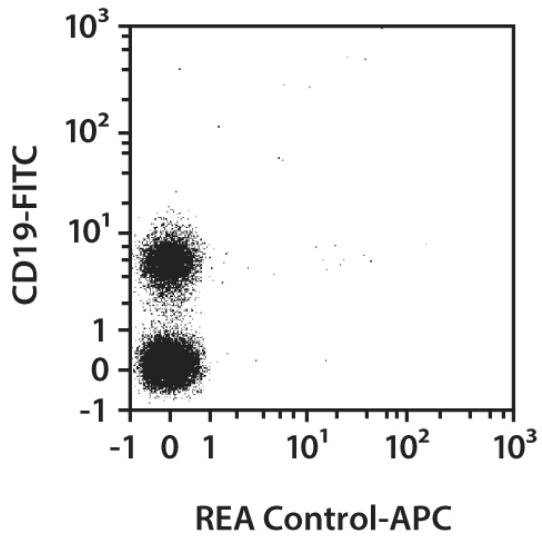
REA Control-PE


CD19-FITC
Anti-IgD-PE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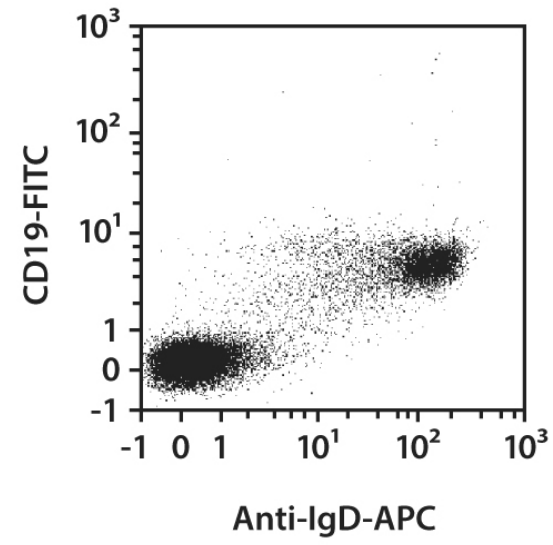
REA Control-APC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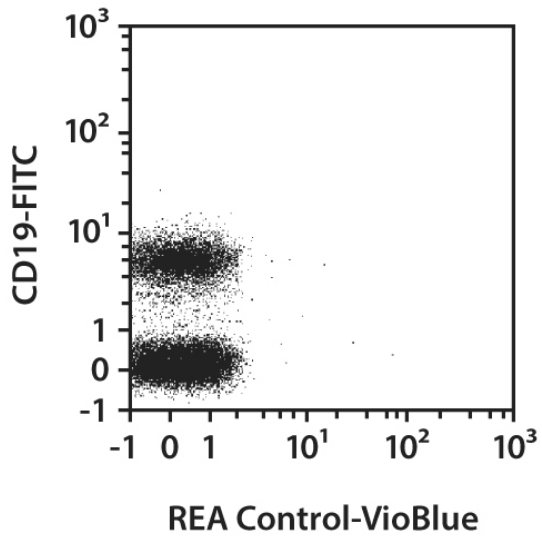
Anti-IgD-APC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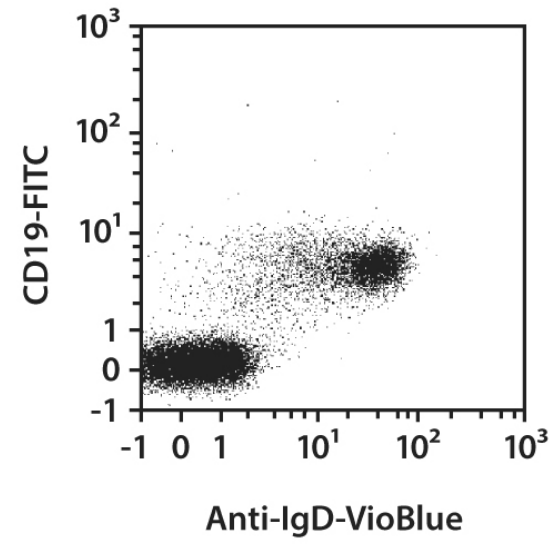
REA Control-VioBlue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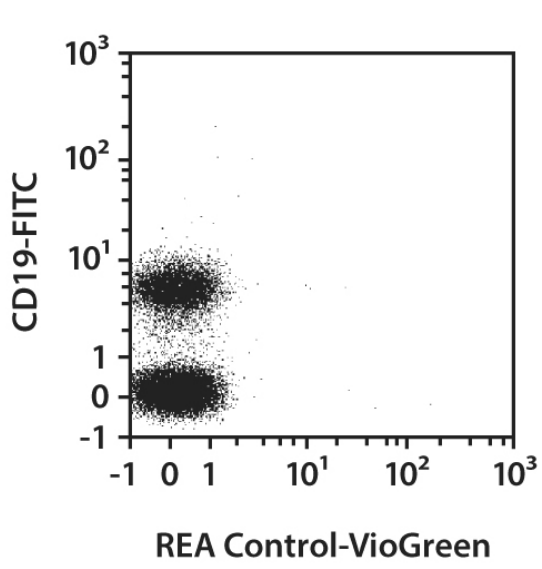
Anti-IgD-VioBlue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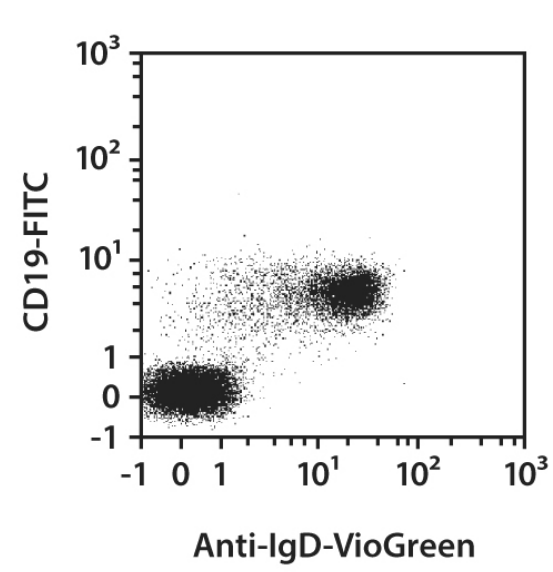
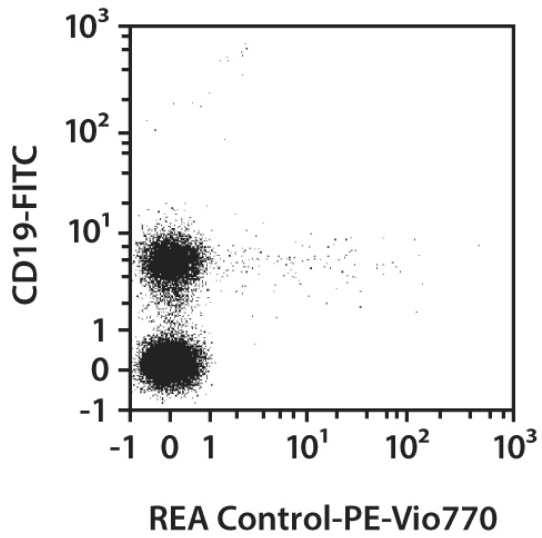
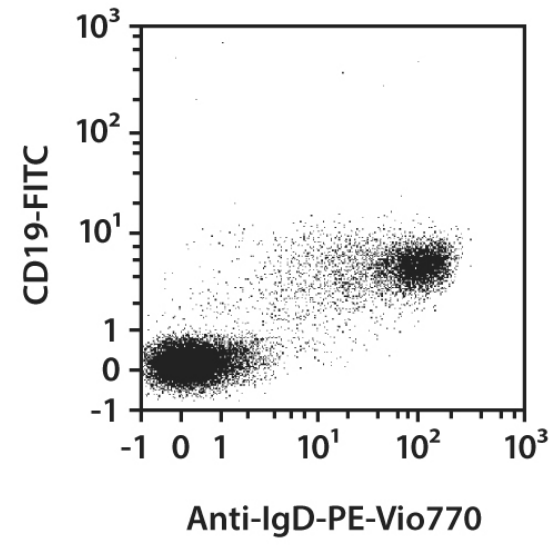
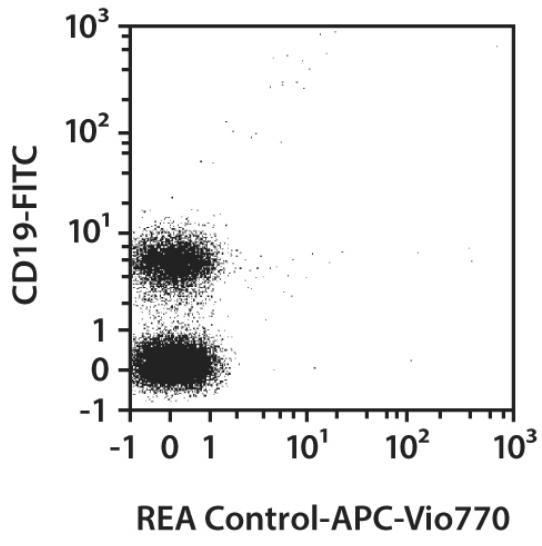
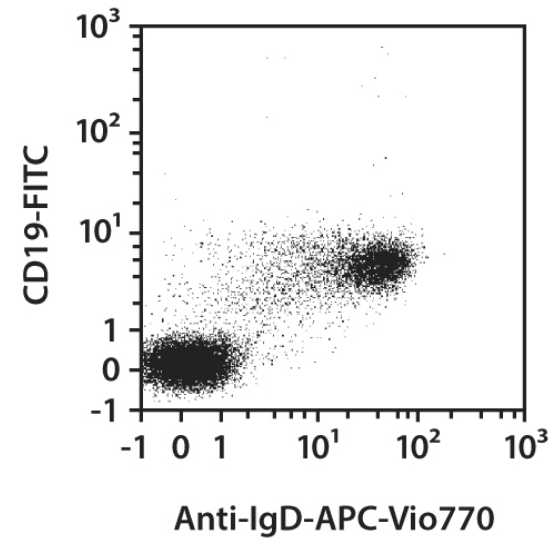
REA Control-VioGreen

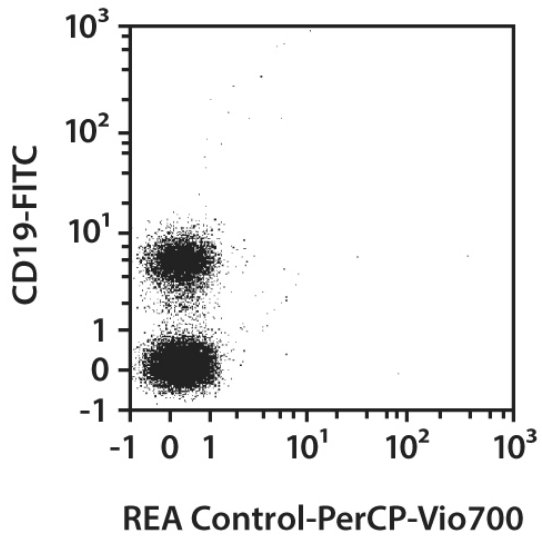

CD19-FITC
Anti-IgD-VioGreen

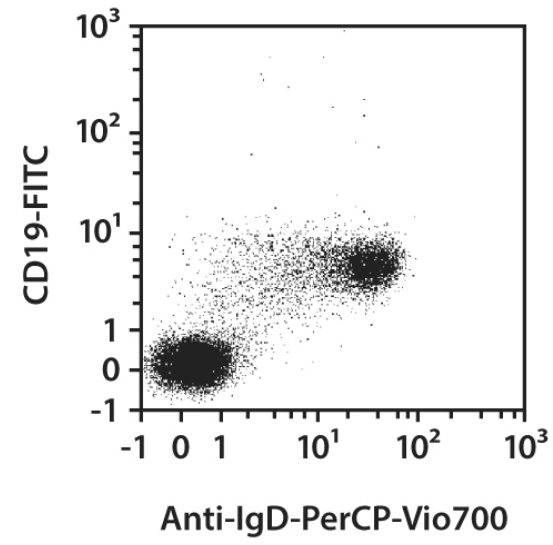


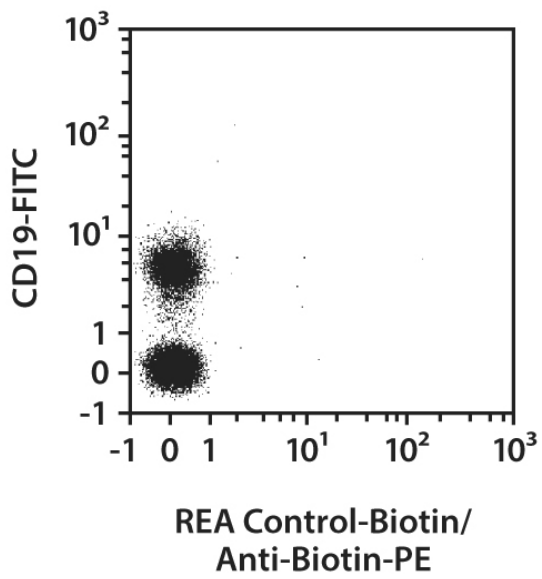



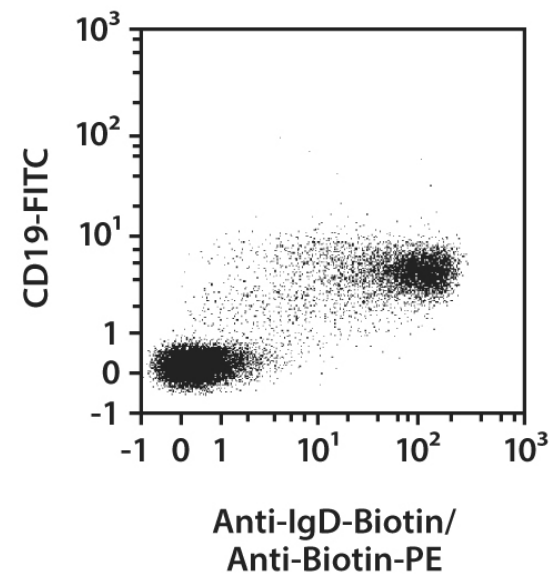

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