Miltenyi Biotec

# Anti-TCRγ/δ Antibodies

**For *in vitro* diagnostic use**

IVD CE

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## 1. General information

**Intended use**
REA591 reacts with human TCRγ/δ. The fluorescently labeled TCRγ/δ antigen can be detected by flow cytometry.

**Reagents and contents**
Monoclonal Anti-TCRγ/δ antibody conjugates

| Product | Σ 100 | Volume | REF |
|---|---|---|---|
| Anti-TCRγ/δ-PE | for 100 tests | 1 mL | 170-078-095 |
| Anti-TCRγ/δ-PE-Vio®770 | for 100 tests | 1 mL | 170-080-029 |

## 2. Technical data and background information

| | |
|---|---|
| **Antigen** | TCRγ/δ |
| **Clone** | REA591 |
| **Isotype** | recombinant human IgG1, κ light chain |
| **Alternative names of antigen** | TCRgd |
| **Product formulation** | Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide. |
| +2°C +8°C | Store at +2 °C to +8 °C. Do not freeze. |
| | Store protected from light. |
| | The use-by date is indicated on the vial label. |
| | For in-use stability at +2 °C to +8 °C storage temperature refer to the use-by date indicated on the vial label. Do not use the reagent after the use-by date. |

**Expression pattern**
REA591 recognizes the human γ/δ T cell receptor (TCR). The T cell receptor is a heterodimeric glycoprotein associated with the CD3 antigen. It consists of a α and a β chain (TCRα/β) or a γ and a δ chain (TCRγ/δ). The γ and δ TCR chains are composed of constant and variable regions, each encoded by distinct gene segments. The γ chain forms either disulfide-linked or non-disulfide–linked heterodimers with the δ-subunit. The γ/δ T cell receptor is present on a subset of T lymphocytes in peripheral blood. TCRγ/δ is involved in the antigen recognition of tumor-associated antigens or bacterial antigens presented by MHC class I molecules. Additional information: Clone REA591 displays negligible binding to Fc receptors.

## 3. Warnings and precautions

- ▲ Analysis results obtained by use of the reagents shall never be the sole basis for classification of disease states.
- ▲ Interpretation of results is under the full responsibility of the user.
- ▲ For all handling, consideration of good laboratory practice (GLP) regulations is recommended.
- ▲ Use of the reagents is restricted to trained and qualified personnel only.
- ▲ All biological specimens and all materials that come into contact with blood and blood products must be treated as infectious material. Regulations for the treatment and disposal of infectious material must be followed.
- ▲ Reagents contain sodium azide ($NaN_3$), a chemical highly toxic in pure form. However, at product concentrations, it is not classified as hazardous. Sodium azide may react with lead and copper plumbing to form highly explosive buildups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing. Safety guidelines must be observed.
- ▲ For material required but not provided the manufacturers recommendations and safety regulations must be followed.

Directions of the package insert must be followed to obtain accurate and reproducible results.

## 4. Application

Reagents can be used for immunophenotyping by flow cytometry. Abnormal numbers of cells expressing this antigen or aberrant expression levels of the antigen can be expected in some disease states. It is important to understand the normal expression pattern for this antigen and its relationship to expression of other relevant antigens in order to perform appropriate analysis.

Expression of TCRγ/δ may be used as aid to diagnostic in the characterization of samples from individuals suspected with hematologic neoplasia.

## 5. Materials required but not provided

- Disposable capped polystyrene tubes, 12×75 mm
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting e.g. MACS® BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (+2 °C to +8 °C).
- Red blood cell (RBC) lysing solution: e.g. Red Blood Cell Lysis Solution (10×), Miltenyi Biotec, (# 170-080-033), or equivalent
- Double-distilled water
- Micropipettes with disposable tips: variable micropipettes with volume ranges of 10–100 µL and 100–1000 µL
- Low speed centrifuge: minimum speed 300×g, with 12×75 mm tube carriers
- Vortex mixer
- Flow cytometer with appropriate laser and filter settings

## 6. Protocol

**Principle of method:**
The antibody reagent provided enables the identification of a specific target cell type by flow cytometry. This technique is based on fluorochrome-conjugated antibodies binding to specific antigens expressed by the target cells. Incubating a sample of interest, e.g., peripheral blood mononuclear cells (PBMC), with the provided antibody reagent leads to fluorescent staining of the cell type expressing the specific target antigen. Analysis of the sample is performed in a flow cytometer at a single-cell level. The analysis is based on the detection of characteristic light emission patterns emitted by the fluorescently labeled antibody upon excitation with laser light. The collected data can be processed and analyzed using flow cytometry software.

**Important notes:**
Under some conditions red blood cells may not lyse within 10 minutes. In this case extend lysis time to 20 minutes before centrifugation of samples.

Exposure of reagents to temperatures below +2 °C and above +8 °C and to light should be minimized during handling.

**Sample requirements**
- Reagents can be used for determination of antigen-positive cells in whole blood samples by flow cytometry. Each cell source can have different storage conditions and limitations that should be considered prior to collection and analysis. For collection of patient samples European and national legislation must be followed.
- Whole blood samples should be stained within 24 hours.
- Viability of the cells should be assessed and use of samples with at least 80% viable cells is suggested in order to minimize risk of erroneous results.

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- Cell count of white blood cells (WBC) should not exceed $5\times10^7$ cells/mL.

  **Note:** If necessary dilute cell sample with PEB buffer.

**Protocol for cell surface staining**

1. Dilute 10× Red Blood Cell Lysis Solution 1:10 with double- distilled water ($ddH_2O$). For example, dilute 1 mL of 10× Red Blood Cell Lysis Solution with 9 mL of $ddH_2O$.

   **Note:** Do not dilute with deionized water. Store prepared 1× Red Blood Cell Lysis Solution at room temperature. Discard unused solution at the end of the day.

2. Add 100 µL of whole blood to a 12×75 mm tube.
3. Add 10 µL of fluorochrome-conjugated antibody to 100 µL of cell sample in a 12×75 mm tube.
4. Mix well and incubate for 15 minutes in the dark at room temperature (+20 °C to +25 °C).

   **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.

5. Add 2 mL of 1× RBC Lysis Solution to each tube. Immediately vortex thoroughly for 3 seconds and incubate for 10 to 20 minutes at room temperature in the dark.
6. Centrifuge at 300×g for 10 minutes. Remove supernatant.
7. Wash cells by adding 1–2 mL of buffer, centrifuge at 300×g for 10 minutes. Remove supernatant.
8. Resuspend cell pellet in a suitable amount of buffer and proceed to flow cytometric analysis. Store samples at +2 °C to +8 °C until analysis.

   **Note:** Minimize exposure of samples to light.

**Quality control**

It is recommended to run regularly a control sample from a normal adult specimen or commercially available whole blood control as a quality control of the system. For excitation and emission data of fluorochrome conjugated reagent please refer to chapter 8.

## 7. Performance characteristics

**Precision**

Anti-TCRγ/δ antibodies were tested by flow cytometry using a lyse-wash protocol on whole blood from healthy donors. Reproducibility was assessed by measuring the frequency of TCRγ/δ positive cells in replicate measurements performed by different operators using the same set of different donor samples. Precision was infered from calculating the mean, standard deviation and coefficient of variation of the frequency of positive cells. All values were within the acceptance criterion.

**Analytical specificity**

Analytical specificity was evaluated by comparing REA591 to a relevant reference clone of the same specificity. Reactivity towards the same antigen was infered from the antibody blocking capacity or the staining diagonal observed during co-incubation of REA591 with the reference clone. Measurements were performed using different donor samples. All measurements were within the acceptance criterion.

## 8. Excitation and emission data of fluorochrome conjugates

| Fluorochrome | Excitation laser (nm) | Excitation maximum (nm) | Emission maximum (nm) |
|---|---|---|---|
| VioBlue® | 405 | 400 | 452 |
| VioGreen™ | 405 | 388 | 520 |
| VioBright™ FITC | 488 | 496 | 522 |
| FITC | 488 | 495 | 520 |
| PE | 488 or 561 | 565 | 578 |
| PE-Vio®615 | 488 or 561 | 565 | 619 |
| PerCP | 488 | 482 | 675 |
| PerCP-Vio®700 | 488 | 482 | 676 |
| PE-Vio®770 | 488 or 561 | 565 | 775 |
| APC | 561 or 635 | 652 | 660 |
| APC-Vio®770 | 561 or 635 | 652 | 775 |

## 9. Limitations

Single color immunofluorescence provides only limited information and is not the method of choice for comprehensive analysis of hematological malignancies. Multicolor immunophenotyping allows more precise definition of atypical cell populations. Therefore, multicolor analysis using relevant combinations of reagents is highly recommended.

As reagents can be used in different combinations, each individual laboratory needs to become familiar with the reactivity of each antibody in conjunction with other markers in normal and abnormal samples. Combinations of recombinant human IgG, κ light chain antibodies with anti-human IgG1 or anti-human Ig κ light chain reagents can generate misleading results.

Use of monoclonal antibodies in patient treatment can interfere with recognition of target antigens by this reagent. This should be considered when analyzing samples from patients treated in this fashion. Miltenyi Biotec has not characterized the effect of the presence of therapeutic antibodies on the performance of this reagent.

Reagent data performance was collected typically with EDTA-treated blood. Reagent performance can be affected by the use of other anticoagulants.

## 10. References


1. Wood BL *et al*: Bethesda International Consensus Recommendatios on the immunpheotypic Analysis of Hematolypmphoid Neoplasia by Flow Cytometry: Optimal Reagents and Reporting for the Flow Cytometric Diagnosis of Hematopoietic Neoplasia. Cytometry Part B 2006; 72B: S14-S22, 2007
2. van Dongen JJM *et al*: EuroFlow antibody panels for standardized m-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. Leukemia 2012; 26: 1908-1975
3. Rothe G, Schmitz G. Consensus protocol for the flow cytometric Immunophenotyping of hematopoietic malignancies. Leukemia1996; 10:877-895
4. Stelzer GT *et al*: U.S.-Canadian Consensus Recommendations on the Immunophenotypic Analysis of Hematologic Neoplasia by Flow Cytometry: Standardization and Validation of Laboratory Procedures. Cytometry 1997; 30: 214–230
5. Clinical and Laboratory Standards Institute (CLSI). Enumeration of Immunologically Defined Cell Populations by Flow Cytometry; Approved Guideline - Second Edition. CLSI document H42-A2 (ISBN 1-56238-640-9) 2007
6. Clinical and Laboratory Standards Institute (CLSI). Clinical Flow Cytometric Analysis of Neoplastic Hematolympoid Cells; Approved Guideline - Second Edition. CLSI document H43-A2 (ISBN 1-56238-635-2) 2007
6. Bonneville, M. *et al.* (2010) Gammadelta T cell effector functions: a blend of innate programming and acquired plasticity. Nat. Rev. Immunol. 10(7): 467–478.
7. Airoldi, I. *et al.* (2015) γδ T-cell reconstitution after HLA-haploidentical hematopoietic transplantation depleted of TCR-αβ$^+$/CD19$^+$ lymphocytes. Blood 125(15): 2349–2358.
8. Wiest, D. L. (2016) Development of γδ T cells, the special-force soldiers of the immune system. Methods Mol. Biol. 1323: 23–32.


## 11. Glossary of symbols