Technical Data Sheet

# PE Rat Anti-Mouse IL-17A

## Product Information

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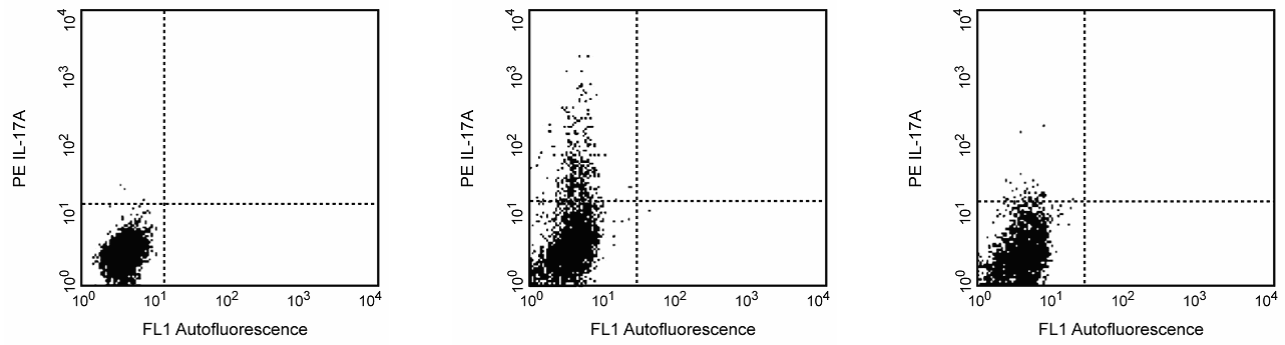
| **Material Number:** | **559502** |
| **Size:** | 0.1 mg |
| **Concentration:** | 0.2 mg/ml |
| **Clone:** | TC11-18H10 |
| **Immunogen:** | Recombinant Mouse IL-17 Protein |
| **Isotype:** | Rat IgG1, κ |
| **Reactivity:** | QC Testing: Mouse |
| **Storage Buffer:** | Aqueous buffered solution containing ≤0.09% sodium azide. |

## Description

The TC11-18H10 antibody reacts with recombinant and natural mouse IL-17A proteins. IL-17A, also known as CTLA-8, is a T cell-derived cytokine that promotes inflammatory responses. Mouse IL-17A is a proinflammatory cytokine that can induce the release of IL-6 by mouse stromal cells. It has been shown to support the growth of hemopoietic progenitors in vitro; it can also stimulate granulopoiesis in vivo. The TC11-18H10 antibody has been reported to neutralize IL-17A activity. Recent studies have shown that IL-17A is produced by a unique subset of Th17 cells that develop along a pathway distinct from the Th1- and Th2- cell differentiation pathways. The mouse IL-17A cDNA was isolated from a cDNA library generated from TCRαβ+CD4-CD8-thymocytes.




***Characterization of IL-17A-producing cells within a stimulated mouse EL4 thymoma cell population.*** *EL4 cells were cultured overnight in the presence of GolgiPlug™ (1 µg/ml final concentration; Cat. No.555029) without (left panel) or with PMA (5 ng/ml final concentration; Sigma, Cat. No.P-8139) and Ionomycin (500 ng/ml final concentration; Sigma, Cat. No.I-0634) (middle and right panels). The cells were fixed, permeabilized and subsequently stained with 0.125 µg of PE conjugated anti-mouse IL-17A (PE-TC11-18H10, Cat. No.559502) using Pharmingen's I/C staining protocol (left and middle panels). To demonstrate the specificity of staining, the binding of PE-TC11-18H10 was blocked by the preincubation of the fixed/permeabilized, stimulated EL4 cells with unlabeled TC11-18H10 antibody (10 µg; Cat. No. 559501, right panel). The quadrant markers for the bivariate dot plots were set based on the autofluorescence control, and verified with the unstimulated stained (left panel) and unlabeled antibody blocking (right panel) controls.*

## Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

## Application Notes

**Application**

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| Intracellular staining (flow cytometry) | Routinely Tested |

**Recommended Assay Procedure:**

**BD Biosciences**

bdbiosciences.com

| United States | Canada | Europe | Japan | Asia Pacific | Latin America/Caribbean |
|---|---|---|---|---|---|
| 877.232.8995 | 888.259.0187 | 32.53.720.550 | 0120.8555.90 | 65.6861.0633 | 55.11.5185.9995 |

For country-specific contact information, visit **bdbiosciences.com/how_to_order/**

**Recommended Assay Procedure:**

**Immunofluorescent Staining and Flow Cytometric Analysis:** The TC11-18H10 antibody is useful for immunofluorescent staining and flow cytometric analysis to identify and enumerate IL-17A producing cells within mixed cell populations. The PE-conjugated TC11-18H10 antibody (Cat. No. 559502) is especially suitable for these experiments (see image). For optimal immunofluorescent staining and flow cytometric analysis, this anti-cytokine antibody should be titrated (≤ 0.25 µg mAb/million cells). For specific methodology, please visit our web site, www.bdbiosciences.com, and go to the protocols section or the chapter on intracellular staining in the Immune Function Handbook..

A useful control for demonstrating the specificity of staining is to pre-block paraformaldehyde-fixed/saponin-permeabilized target cells with unlabeled TC11-18H10 antibody (Cat. No. 559501) prior to staining. The intracellular staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe. A suitable rat IgG1 isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized mouse is PE-R3-34 (Cat. No. 554685); use at comparable concentrations to antibody of interest (e.g., ≤ 0.25 µg mAb/1 million cells).

**OTHER APPLICATIONS**

**1. ELISA Capture:** The purified TC11-18H10 antibody (Cat. No. 555068) is useful as a capture antibody for a sandwich ELISA for measuring mouse IL-17A protein levels. Purified TC11-18H10 antibody can be paired with the biotinylated TC11-8H4 (Cat. No.555067) antibody as the detecting antibody, with recombinant mouse IL-17A protein as the standard. The purified TC11-18H10 antibody should be titrated from 0.5 µg/ml to 2.0 µg/ml to determine its optimal concentration for ELISA capture. To obtain linear standard curves, doubling dilutions of mouse IL-17A ranging from ~2,000 to 15 pg/ml are recommended for inclusion in each ELISA plate. For specific methodology, please visit our web site, www.bdbiosciences.com, and go to the protocols section or the chapter on ELISA in the Immune Function Handbook.

**2. Western blotting:** The TC11-1810 antibody has been found useful for Western Blotting. Please note that this application is not routinely tested at BD Biosciences Pharmingen.

**3. Neutralization:** TCII-18H10 has been shown to be a neutralizing antibody. Please note that this application is not routinely tested at BD Biosciences.

## Suggested Companion Products

| Catalog Number | Name | Size | Clone |
|---|---|---|---|
| 555029 | Protein Transport Inhibitor (Containing Brefeldin A) | 1.0 ml | (none) |
| 554685 | PE Rat IgG1, κ Isotype Control | 0.1 mg | R3-34 |
| 555028 | BD Cytofix/Cytoperm Plus Kit (with BD GolgiPlug) | 250 tests | (none) |

## Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/pharmingen/colors.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
4. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

## References


Kennedy J, Rossi DL, Zurawski SM, et al. Mouse IL-17: a cytokine preferentially expressed by alpha beta TCR + CD4-CD8-T cells. *J Interferon Cytokine Res.* 1996; 16(8):611-617.(Biology)

Prussin C, Metcalfe DD. Detection of intracytoplasmic cytokine using flow cytometry and directly conjugated anti-cytokine antibodies. *J Immunol Methods.* 1995; 188(1):117-128.(Methodology: IC/FCM Block)

Schwarzenberger P, La Russa V, Miller A, et al. IL-17 stimulates granulopoiesis in mice: use of an alternate, novel gene therapy-derived method for in vivo evaluation of cytokines. *J Immunol.* 1998; 161(11):6383-6389.(Biology)