Technical Data Sheet **Alexa Fluor® 647 Rat anti-Mouse IL-17A**

Product Information

| Material Number: | 560224 | | |
|------------------|--|--|--|
| Size: | 25 μg | | |
| Concentration: | 0.2 mg/ml | | |
| Clone: | TC11-18H10 | | |
| Immunogen: | Recombinant Mouse IL-17A Protein | | |
| Isotype: | Rat (LEW) 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 TCR $\alpha\beta$ +CD4-CD8- thymocytes.



Characterization of IL-17A-producing cells within a stimulated mouse EL4 thymoma cell population. EL4 cells were stimulated (left and middle panels) or unstimulated (right panel) with PMA (50 ng/ml final concentration; Sigma, Cat. No.P-8139) and lonomycin (1000 ng/ml final concentration; Sigma, Cat. No.P-8139) and lonomycin (1000 ng/ml final concentration; Sigma, Cat. No.P-8139) and lonomycin (1000 ng/ml final concentration; Sigma, Cat. No.P-8139) and lonomycin (1000 ng/ml final concentration; Sigma, Cat. No.P-8139) and lonomycin (1000 ng/ml final concentration; Sigma, Cat. No.I-0634) in the presence of BD GolgiStop™ (Cat. No. 554724). The cells were fixed, permeabilized and subsequently stained with Alexa 647 conjugated anti-mouse IL-17A (clone TC11-18H10, Cat. No.560184/560224) (middle and right panels) or Alexa 647 rat IgG1 isotype control (clone R3-34, Cat. no. 557731) (left panel) using Pharmingen's I/C staining protocol. The quadrant markers for the bivariate dot plots were set based on the autofluorescence control, and verified with the unstimulated stained (right panel) and non-specific antibody (left panel) controls.

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

Application Notes

| Application | | | |
|---|------------------|--|--|
| Intracellular staining (flow cytometry) | Routinely Tested | | |

Recommended Assay Procedure:

For flow cytometry of cell suspensions from peripheral lymphoid tissues, it is recommended that the cells be pre-incubated with Mouse BD Fc BlockTM purified anti-mouse CD16/CD32 mAb 2.4G2 (Cat. No.553141/553142).

Suggested Companion Products

| Catalog Number | Name | Size | Clone | |
|----------------|--|-----------|--------|--|
| 557731 | Alexa Fluor® 647 Rat IgG1, κ Isotype Control | 0.1 mg | R3-34 | |
| 553141 | Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™) | 0.1 mg | 2.4G2 | |
| 554715 | BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop) | 250 tests | (none) | |

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Product Notices

- 1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
- 2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
- 3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
- 4. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
- 5. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
- 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.
- 7. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.

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)

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)