Technical Data Sheet

PerCP-Cy™5.5 Mouse anti-Human IL-17F

Product Information

Material Number:	561336
Alternate Name:	Interleukin-17F; cytokine ML-1; ML-1; IL-24
Size:	25 tests
Vol. per Test:	5 µl
Clone:	O33-782
Immunogen:	Human IL-17F
Isotype:	Mouse IgG1, ĸ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and $\leq 0.09\%$ sodium azide.

Description

The O33-782 monoclonal antibody specifically binds to Interleukin-17F (IL-17F). IL-17F is a member of the IL-17 family of cytokines. IL-17F is encoded by the *IL17F* gene located in chromosome 6 (location: 6p12). IL-17F is a proinflammatory cytokine that is produced by activated T cells including differentiated CD4+ T helper 17 (Th17) cells. Activated Th17 cells can express disulfide-linked IL-17F and IL-17A homodimers as well as IL-17A/IL-17F heterodimers. These IL-17 dimers act by binding to and signaling through IL-17 receptor complexes (IL-17R). IL-17R are comprised of transmembrane IL-17RA and IL-17-RC protein subunits that are expressed by a variety of target cells including epithelial and endothelial cells, keratinocytes, fibroblasts, and granulocytes. IL-17F can induce target cells to produce proinflammatory cytokines such as IL-1 β , IL-6, G-CSF, GM-CSF, and TNF and chemokines including CXCL1/Gro- α , CXCL2/Gro- β , and CXCL8/IL-8 that attract and activate leukocytes, eg, neutrophils. Th17 and other IL-17F-producing cells play protective roles in the clearance of extracellular pathogens, including bacteria and fungi. IL-17F can also play adverse roles in inflammation associated with asthma and autoimmune diseases.



Flow cytometric analysis of IL-17F expression by resting or activated human peripheral blood mononuclear cells (PBMC). Human PBMC were either unstimulated (Left Panels) or stimulated with Phorbol 12-Myristate 13-Acetate (PMA; Sigma P-8139) plus lonomycin (Sigma; I-0634) in the presence of BD GolgiStop[™] Protein Transport Inhibitor (Cat. No. 554724) for 5 hours (Middle Panels) or cultured in Th17 polarization conditions and restimulated with PMA and lonomycin in the presence of BD GolgiStop[™] for 5 hours (Right Panels). Cells were then fixed and permeabilized using BD Cytofix/Cytoperm[™] reagents (Cat. No. 554714) followed by staining with PerCP-Cy[™] 5.5 Mouse anti-Human IL-17F (Cat. No. 561336), PE Mouse anti-Human CD4 (Cat. No. 555347), FITC Mouse Anti-Human IFN-γ (Cat. No. 554700) and Alexa Fluor® 647 Mouse anti-Human IL-17A (Cat. No. 560490). Two-color flow cytometric dot plots showing the correlated expression patterns of IL-17F versus IL-17A or IFN-γ were derived from CD4+ gated events with the forward and side light-scatter characteristics of intact lymphocytes. Flow cytometry was performed using a BD[™] LSR II Flow Cytometry System. Other compatible fixation and permeabilization treatments are listed in the "Recommended Assay Procedure."

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Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography. The antibody was conjugated with PerCP-Cy5.5 under optimum conditions, and unconjugated antibody and free PerCP-Cy5.5 were removed. Storage of PerCP-Cy5.5 conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity. Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

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Recommended Assay Procedure:

This antibody conjugate is suitable for intracellular staining of human peripheral blood mononuclear cells using BD Cytofix/CytopermTM reagents, BD PharmingenTM Human FoxP3 Buffer Set or BDTM Phosflow fixation and permeabilization buffers (Fix buffer I with Perm/Wash Buffer I, Perm Buffer II, or Perm Buffer III).

Suggested Companion Products

Catalog Number	Name	Size	Clone
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
554714	BD Cytofix/Cytoperm [™] Fixation/Permeablization Kit	250 tests	(none)
555347	PE Mouse Anti-Human CD4	100 tests	RPA-T4
554700	FITC Mouse Anti-Human IFN-γ	0.1 mg	B27
560490	Alexa Fluor® 647 Mouse anti-Human IL-17A	100 tests	N49-653
560098	Human FoxP3 Buffer Set	100 tests	(none)
557870	Fix Buffer I	250 ml	(none)
557885	Perm/Wash Buffer I	125 ml	(none)
558052	Perm Buffer II	125 ml	(none)
558050	Perm Buffer III	125 ml	(none)

Product Notices

- 1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1×10^{6} cells in a 100-µl experimental sample (a test).
- 2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
- 3. An isotype control should be used at the same concentration as the antibody of interest.
- 4. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
- 5. PerCP-Cy5.5–labelled antibodies can be used with FITC- and R-PE–labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.
- 6. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
- 7. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
- 8. 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.
- 9. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
- 10. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
- 11. This PerCP-conjugated product is sold under license to the following patent: US Patent No. 4,876,190.
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References

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