Technical Data Sheet

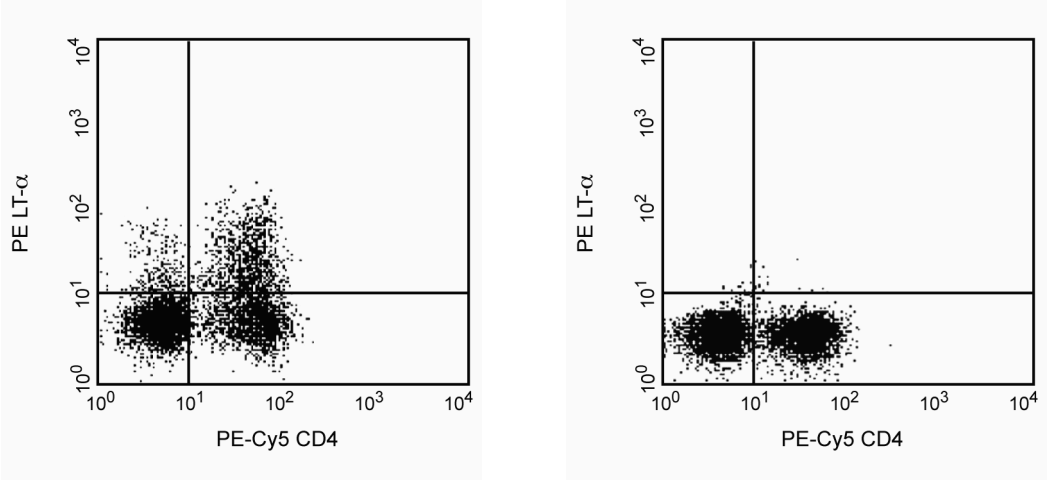
PE Mouse Anti-Human LT-α

Product Information

Material Number:	**554556**
Alternate Name:	TNF-β
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	359-81-11
Immunogen:	Recombinant Human LT-α (TNF-β)
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The 359-81-11 antibody reacts with human Lymphotoxin-α (LT-α, also known as tumor necrosis factor-β or TNF-β). The immunogen used to generate the 359-81-11 hybridoma was recombinant human LT-α. This is a neutralizing antibody.



Detection of LT-α expression by stimulated human lymphocytes using PE-359-81-11. *Human PBMC were stimulated with soluble anti-human CD3 mAb (1 µg/ml, clone UCHT1, Cat. No. 555329) and recombinant human IL-2 (10 ng/ml, Cat. No. 554603) for 2 days. The cells were subsequently cultured in medium containing recombinant human IL-2 for 3 days. Finally, the cells were harvested and re-stimulated for 6 hr with immobilized anti-human CD3 mAb (10 µg/ml) and soluble anti-human CD28 (20 ng/ml, clone CD28.2, at. No. 555725) in the presence of 2 µM GolgiStop™ (aka, monensin; Cat. No. 554724). The cells were harvested, stained with PE-Cy5™-anti-CD4 (Cat. No. 555348), fixed, permeabilized, and subsequently stained with 0.25 µg of PE- anti-human LT-α antibody by using the BD Pharmingen™ staining protocol (left panel). To demonstrate specificity of staining, the binding by PE-359-81-11 was blocked by each of the following: 1) preincubation of the conjugated antibody with excess recombinant human LT-α (Cat. No. 554619; right panel) and by 2) preincubation of the fixed/permeabilized cells with unlabeled 359-81-11 mAb (data not shown) prior to staining with the PE- 359-81-11. The quadrant markers for the bivariate dot plots were set based on the autofluorescence controls and verified using the recombinant cytokine blocking and unlabeled antibody blocking specificity 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 by gel filtration chromatography.

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

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

BD Biosciences

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Immunofluorescent Staining and Flow Cytometric Analysis: The 359-81-11 antibody is useful for intracellular immunofluorescent staining and flow cytometric analysis to identify and enumerate LT-α producing cells within mixed cell populations. PE-conjugated 359-81-11 antibody is especially suitable for these studies. The use of a specificity control, such as one of the following, is suggested: 1) recombinant human TNF-β (Cat No. 554619), 2) unlabeled 359-81-11 antibody (Cat. No. 554554), or 3) mouse IgG1 isotype control, PE-MOPC-21 (Cat. No. 554680).

ELISA Detection: The biotinylated 359-81-11 antibody is useful as a detection antibody for a sandwich ELISA for measuring human LT-α protein levels in cell culture supernatants. Biotinylated 359-81-11 antibody can be paired with the purified 359-238-8 antibody (Cat. No. 554557) as the capture antibody, with recombinant LT-α (Cat. No. 554619) as the standard. For specific methodology, please visit the protocols section or chapter on ELISA in the Immune Function Handbook, both of which are posted on our web site, www.bdbiosciences.com.

Neutralization: The NA/LE™ 359-81-11 antibody has been reported to be useful for neutralization of human LT-α bioactivity. A suitable NA/LE mouse IgG1 isotype control to match the 359-81-11 antibody is the 107.3 antibody, (Cat. No. 554721).

Suggested Companion Products

Catalog Number	Name	Size	Clone
554680	PE Mouse IgG1, κ Isotype Control	0.1 mg	MOPC-21
555329	Purified NA/LE Mouse Anti-Human CD3	0.5 mg	UCHT1
554603	Recombinant Human IL-2	5 µg	(none)
555725	Purified NA/LE Mouse Anti-Human CD28	0.5 mg	CD28.2
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
555348	PE-Cy™5 Mouse Anti-Human CD4	100 tests	RPA-T4
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)

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. 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

Meager A, Parti S, Leung H, et al. A two-site sandwich immunoradiometric assay of human lymphotoxin with monoclonal antibodies and its applications. *J Immunol Methods.* 1987; 104(2):31-42.(Clone-specific: ELISA, Neutralization)

Meager A, Parti S, Leung H, Peil E, Mahon B. Preparation and characterization of monoclonal antibodies directed against antigenic determinants of recombinant human tumour necrosis factor (rTNF). *Hybridoma.* 1987; 6(3):305-312.(Clone-specific: ELISA, Neutralization)

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)