Technical Data Sheet

PE Hamster Anti-Rat/Mouse TNF

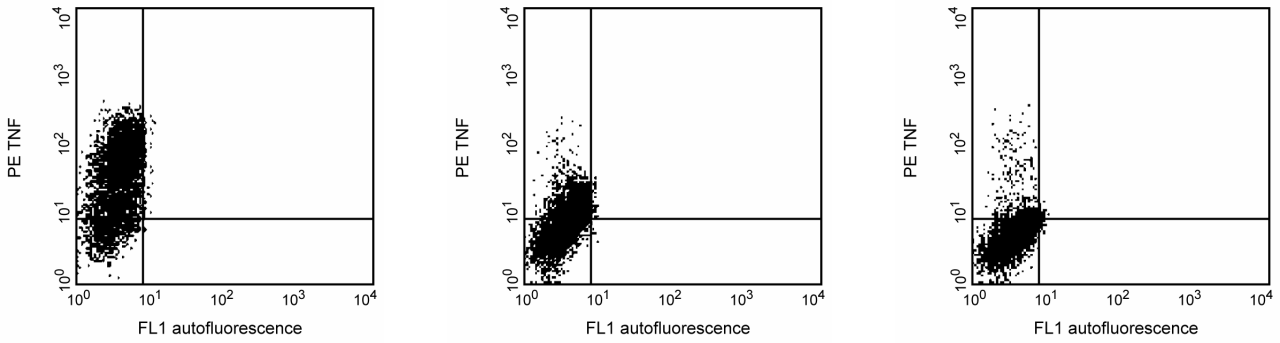
Product Information

Material Number:	**559503**
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	TN3-19.12
Immunogen:	Recombinant mouse TNF protein
Isotype:	Armenian Hamster IgG1, λ1
Reactivity:	QC Testing: Rat, Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The TN3-19.12 antibody reacts with rat and mouse tumor-necrosis factor (TNF) proteins (also known as TNF-α) . Moreover, the TN3-19.12 antibody is reported to crossreact with rabbit TNF, but it does not crossreact with mouse lymphotoxin-α (LT-α, also known as TNF-β) nor with human TNF. The immunogen used to generate the TN3-19.12 hybridoma was E. coli-expressed, purified recombinant mouse TNF protein. This monoclonal antibody has been reported to neutralize the bioactivities of mouse, rat and rabbit TNF.

This antibody is routinely tested by flow cytometric analysis. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.



Expression of TNF by RiCK-2 Positive control cells. *In vitro differentiated and re-stimulated LOU lymphocytes (RiCK-2 Cells, Cat. No. 555094) were fixed, permeabilized, and subsequently stained with PE-conjugated rat anti-mouse TNF antibody (PE-TN3-19.12, Cat. No. 559503) by using Pharmingen's staining protocol (see Figure, left panel). To demonstrate specificity of staining, the binding of PE-TN3-19.12 was blocked by the preincubation of the conjugated antibody with molar excess of recombinant rat TNF (0.25 µg, Cat. No. 555109; middle panel), and by preincubation of the fixed/permeabilized cells with an excess of the unlabeled TN3-19.12 mAb (5µg, Cat. No. 557516; right panel). The quadrant markers for the bivariate dot plots were set based on the autofluorescence control, and verified with the recombinant cytokine blocking (middle panel) 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

Recommended Assay Procedure:

The PE-conjugated TN3-19.12 antibody can be used for multicolor immunofluorescent staining and flow cytometric analyses to identify and enumerate rat TNF-producing cells within mixed cell populations (see Figure). For optimal immunofluorescent staining with flow cytometric analysis, this anti-cytokine antibody should be titrated (≤ 0.5 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.

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A useful control for demonstrating specificity of staining is either of the following: 1) pre-block the conjugated TN3-19.12 antibody with a molar excess of ligand prior to staining, or 2) pre-block the fixed/permeabilized cells with unconjugated TN3-19.12 antibody (Cat. No. 557516) prior to staining. The staining technique and blocking controls are described in detail by C. Prussin and D. Metcalfe. A suitable hamster IgG1, λ isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized mouse and human cells is PE-G235-2356 (Cat. No. 554711); use at comparable concentrations to antibody of interest (e.g., ≤ 0.5 µg mAb/ 1 million cells).

OTHER APPLICATIONS

In vitro neutralization: The NA/LE™ format of the TN3-19.12 antibody (Cat. No. 557370) is useful for neutralization of mouse TNF bioactivity.

IP/WB: The TN3-19.12 antibody has been reported to be useful for immunoprecipitation and Western blot. Please note that this application is not routinely tested at BD Biosciences Pharmingen.

ELISA: The purified TN3-19.12 antibody (Cat. No. 557516) can be used as a capture antibody for a sandwich ELISA that measures TNF protein levels.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554711	PE Hamster IgG1, λ1 Isotype Control	0.1 mg	G235-2356
555109	Recombinant Rat TNF	5 µg	(none)
557516	Purified Hamster Anti-Mouse/Rat TNF	0.5 mg	TN3-19.12
555094	RiCK-2 Cytokine Positive Control Cells	5x10^6 cells	(none)
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. 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/pharmingen/colors.
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

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Merrick BA, He CY, Craig WA, et al. Two dimensional gel electrophoresis of cellular and secreted proteins from rat alveolar macrophages after lipopolysaccharide treatment. *Appl Theor Electrophor.* 1992; 2(6):177-187.(Clone-specific: Immunoprecipitation, Western blot)

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)

Rabinovici R, Bugelski PJ, Esser KM, et al. Tumor necrosis factor-alpha mediates endotoxin-induced lung injury in platelet activating factor-primed rats. *J Pharmacol Exp Ther.* 1993; 267(3):1550-1557.(Clone-specific: Neutralization)

Sheehan KC, Ruddle NH, Schreiber RD. Generation and characterization of hamster monoclonal antibodies that neutralize murine tumor necrosis factors. *J Immunol.* 1989; 142(11):3884-3893.(Immunogen: ELISA, Immunoprecipitation, Neutralization, Western blot)

Takahashi S, Kapas L, Fang J, Krueger JM. An anti-tumor necrosis factor antibody suppresses sleep in rats and rabbits. *Brain Res.* 1995; 690(2):241-244.(Clone-specific: Neutralization)