Technical Data Sheet

V450 Rat Anti-Mouse TNF

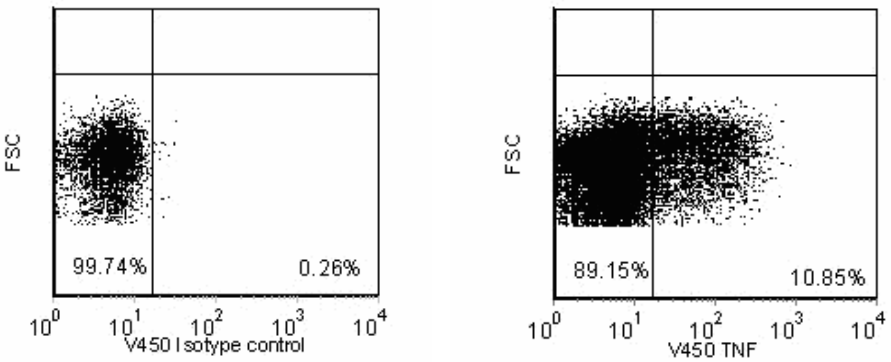
Product Information

Material Number:	**560655**
Size:	50 µg
Concentration:	0.2 mg/ml
Clone:	MP6-XT22
Immunogen:	Recombinant mouse TNF
Isotype:	Rat IgG1
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing protein stabilizer and ≤0.09% sodium azide.

Description

The MP6-XT22 antibody reacts with mouse tumor necrosis factor (TNF, also known as TNF-α). The immunogen used to generate this hybridoma was recombinant mouse TNF.

The antibody is conjugated to BD Horizon™ V450, which has been developed for use in multicolor flow cytometry experiments and is available exclusively from BD Biosciences. It is excited by the **V**iolet laser Ex max of 406 nm and has an Em Max at **450** nm. Conjugates with BD Horizon™ V450 can be used in place of Pacific Blue™ conjugates.



Flow cytometric analysis for TNF in activated mouse splenocytes. *Mouse Intracellular Cytokine-1 positive control cells (MiCK-1) offered by BD Biosciences as MN 554652, are activated mouse splenocytes prepared in the presence of a protein transport inhibitor. Fixed and permeabilized MiCK-1 cells were stained either with a BD Horizon™ V450 Rat IgG1, κ isotype control (left panel) or with the BD Horizon™ Rat Anti-Mouse TNF antibody (right panel). Dot plots were derived from gated events based on light scattering characteristics for lymphocytes. Flow cytometry was performed on a BD™ LSR II flow cytometry system.*

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 with BD Horizon™ V450 under optimum conditions, and unreacted BD Horizon™ V450 was removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Flow cytometry: The MP6-XT22 antibody is useful for immunofluorescent staining and flow cytometric analysis to identify and enumerate TNF producing cells within mixed cell populations. A useful control investigators may consider using for demonstrating specificity of staining, is to pre-block with one of the following reagents: (1) recombinant mouse TNF (Cat. No. 554589) or (2) unlabeled MP6-XT22 antibody (Cat. No. 554416), prior to staining.

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Cell Preparation: Investigators not wishing to utilize MiCK-1 cells may alternatively prepare mouse splenocytes (e.g BALB/c) stimulated for 4-6 hours with PMA (5 ng/mL, Sigma-Aldrich Cat. No. P-8139) and ionomycin (500 ng, Sigma-Aldrich Cat. No. I-0634) in the presence of 1 µg/mL Brefeldin A (BD GolgiPlug™ MN 555029). Investigators are advised to fix and permeabilize the cells prior to staining.

Suggested Companion Products

Catalog Number	Name	Size	Clone
560535	V450 Rat IgG1, κ Isotype Control	0.1 mg	R3-34
554652	MiCK-1 Mouse Cytokine Positive Control Cells	1.0 ml	(none)
553142	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.5 mg	2.4G2
554416	Purified Rat Anti-Mouse TNF	0.1 mg	MP6-XT22
555028	BD Cytofix/Cytoperm Plus Kit (with BD GolgiPlug)	250 tests	(none)
555029	Protein Transport Inhibitor (Containing Brefeldin A)	1.0 ml	(none)
554589	Recombinant Mouse TNF	10 µg	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
3. BD Horizon™ V450 has a maximum absorption of 406 nm and maximum emission of 450 nm. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
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.
5. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
7. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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