Technical Data Sheet

Purified Rat Anti-Mouse TNF

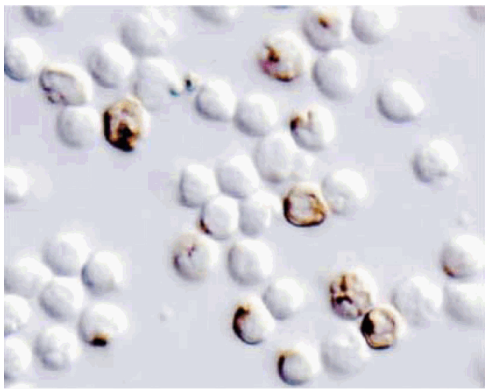
Product Information

Material Number:	**559064**
Size:	0.25 mg
Concentration:	0.5 mg/ml
Clone:	MP6-XT22
Immunogen:	Recombinant mouse TNF
Isotype:	Rat IgG1
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤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.

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



***TNF Staining**: RBC-lysed BALB/c splenocytes were cultured with PMA (Sigma, 5 ng/ml) and ionomycin (Sigma, 500 ng/ml) with GolgiPlug™ (Cat. No. 555029) for 4 hr at 37°C. The activated cells were harvested and the presence of TNF producing cells was detected by immunocytochemistry using a three-step staining procedure that employs a Biotin Goat anti-Rat IgG secondary antibody (Cat. No. 559286) and a horseradish peroxidase-based detection system (Nomarski optics, original magnification 400 X). To demonstrate specificity of staining the binding of the MP6-XT22 (Cat. No. 559064) antibody was blocked by the preincubation of the purified antibody with excess recombinant mouse TNF (Cat. No. 554589; data not shown).*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at 4° C.

Application Notes

Application

Immunocytochemistry (cytospins)	Routinely Tested

Recommended Assay Procedure:

Immunocytochemistry: The ICC format of the purified MP6-XT22 (Cat. No. 559064) antibody can be used to identify and enumerate TNF producing cells by immunocytochemistry. For optimal indirect immunocytochemical staining, the MP6XT22 antibody should be titrated (≤ 1 µg) and visualized via a three-step staining procedure using Biotin Goat Anti-Rat IgG and streptavidin horseradish peroxidase (HRP). A detailed protocol for the procedure is found below. For optimal detection of cytokine producing cells, horseradish peroxidase as the preferred enzyme system.

CYTOKINE IMMUNOCYTOCHEMISTRY PROTOCOL

REAGENTS REQUIRED

1. Fixation Buffer: BD Pharmingen™ ICC Fixation Buffer (BD Cat. No. 550010) or 5% formalin (10% formalin, CMS, Cat. No. 245-684) is dissolved in phosphate buffered-saline (PBS) (Bacto FA Buffer, Difco Laboratories, Cat. No. 2314-15-0)
2. Endogenous Peroxidase Blocking Buffer: DAKO Peroxidase Blocking Reagent (DAKO, Cat. No. S2001).
3. Endogenous Biotin Blocking Buffer: Biotin/Avidin Blocking Kit (Vector Laboratories, Cat. No. SP-2001).

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4. Antibody dilution buffer: BD Pharmingen™ Antibody Diluent for IHC, Cat. No. 559148, supplemented with saponin
5. Microscopic slides: Adhesion Slides (Erie Scientific Company, Cat. No. ER-202B-AD) or for cytospins, Colorfrost /Plus slides (Fisher, Cat. No. 12-550-17).
6. Biotin Goat anti-Rat IgG (Cat. No. 559286), or Anti-Rat Ig HRP Detection Kit (Cat. No. 551013).
7. Detection system: BD Pharmingen™ Streptavidin-HRP, (Cat. No. 550946), or Anti-Rat Ig HRP Detection Kit (Cat. No. 551013).
8. Mounting medium for short-term storage: Aqua-mount® (Lerner Laboratories, Cat. No. 13800).
9. DAB Substrate Kit (contains 3-3 -Diaminobenzidine tetra hydrochloride), (BD Cat. No. 550880), or Anti-Rat Ig HRP Detection Kit (Cat. No. 551013).

PROCEDURE FOR IMMUNOCYTOCHEMICAL STAINING OF SINGLE-CELL PREPARATIONS
This procedure describes the immunoenzymatic technique of staining cytokines within individual cells that are immobilized on microscopic slides via adherence (adherent slides) or centrifugation (cytospins).

ADHESION SLIDES
1. Harvest cells and wash them twice in PBS using centrifugation (400 x g for 5 min) to remove residual protein.
2. Adjust the cell concentration at 4-5 x 10e6 cells/ml in PBS.
3. Place 20 µl of the cell suspension in each well of the adhesion slides and let them adhere at room temperature (RT) for 20 min. Please note that the slides should be washed in PBS at RT for 5 min before transferring the cells.
4. Fix cells on slides using fixation buffer (Cat. No. 550010) for 15 min at RT.
5. Wash slides 2X in PBS with 5 min incubations.
6. Block slides with PBS supplemented with 1% (w/v) BSA (Sigma, Cat. No. A43-78) for 30 min at RT or 10 min at 37°C.
7. Wash slides 2X in PBS and proceed with staining or air dry them and store them at -80°C for future use.
8. Incubate slides with 20 µl of 1% goat serum and PBS with 0.1% (w/v) saponin for 30 min at RT.
9. Wash slides 2X with PBS with 5 min incubations.
10. Block endogenous peroxidase activity with Endogenous Peroxidase Blocking Buffer (20 µl/well) for 10 min at RT.
11. Wash 2X in PBS with 5 min incubations.
12. Incubate each well with Avidin (20 µl/well) for 15 min.
13. Wash 2X in PBS with 5 min incubations.
14. Incubate each well with Biotin (20 µl/well) for 15 min.
15. Wash 2X in PBS with 5 min incubations.
16. Incubate each well for 1 hr at RT with 20 µl of purified cytokine-specific antibody or appropriate immunoglobulin isotype control diluted in Pharmingen's IHC Diluent Buffer (Cat. No. 559148), supplemented with saponin.
17. Wash slides 2X in PBS with 5 min incubations.
18. Incubate each well with 20 µl of a biotinylated secondary antibody diluted in IHC Diluent Buffer for 30 min at RT.
19. Wash 2X in PBS with 5 min incubations.
20. Apply 20 µl of Streptavidin-HRP (BD Cat. No. 550946) to each well on slides and incubate for 30 min at RT.
21. Wash slides 2X with PBS with 5 minutes incubations.
22. Incubate with DAB Substrate as directed, (BD Cat. No. 550880) for less than 5 min at RT.
23. Stop the development of the color reaction by washing with PBS.
24. The slides are subsequently mounted in short-term storage mounting medium.

CYTOSPINS
1. Assemble the Cytospin's sample chamber (e.g. Cytospin 3, Shandon, UK or comparable centrifuge), filter card, slide and cytospin racks according to manufacturer's specifications.
2. Load 40 µl of approximately 1 x 10e6 cells to each sample chamber.
3. Spin slides at 600 rpm for 2 min.
4. Take slides out of the cytospin rack and place them on a staining rack.
5. For fixation and staining please follow the steps 4 through 24 specified above for staining cells on adhesion slides.

Suggested Companion Products

Catalog Number	Name	Size	Clone
559286	Biotin Polyclonal Goat Anti-Rat IgG	0.5 mg	Polyclonal
550010	ICC Fixation Buffer	100 ml	(none)
551013	Anti-Rat Ig HRP Detection Kit	200 tests	(none)
559072	Purified Rat IgG1, κ Isotype Control	0.25 mg	R3-34

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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. 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

Abrams J. Immunoenzymetric assay of mouse and human cytokines using NIP-labeled anti-cytokine antibodies. In: Coligan J, Kruisbeek A, Margulies D, Shevach E, Strober W, ed. *Current Protocols in Immunology.* New York: John Wiley and Sons; 1995:6.20-6.21.(Biology)

Abrams JS, Roncarolo MG, Yssel H, Andersson U, Gleich GJ, Silver JE. Strategies of anti-cytokine monoclonal antibody development: immunoassay of IL-10 and IL-5 in clinical samples. *Immunol Rev.* 1992; 127:5-24.(Biology)

Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J Histochem Cytochem.* 1981; 29(4):577-580.(Methodology)

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