

Technical Data Sheet

Purified Rat Anti-Mouse IFN- γ

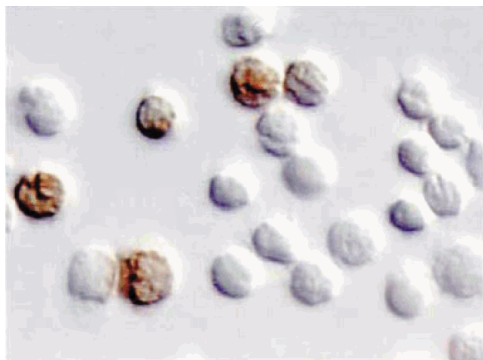
Product Information

Material Number:	559065
Size:	0.25 mg
Concentration:	0.5 mg/ml
Clone:	XMG1.2
Immunogen:	Mouse IFN- γ
Isotype:	Rat IgG1, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing $\leq 0.09\%$ sodium azide.

Description

The XMG1.2 antibody reacts with mouse interferon- γ (IFN- γ) protein. IFN- γ is a pleiotropic cytokine, of approximately 15-17 kDa, involved in the regulation of the immune response. It plays an important role in activation, growth, and differentiation of T and B lymphocytes, macrophages, NK cells and other non-hematopoietic cell types. IFN- γ production is associated with the Th-1 differentiation. This is a neutralizing antibody

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



RBC-lysed BALB/c splenocytes were cultured with PMA (Sigma, Cat. No. P-8139, 5 ng/ml) and ionomycin (Sigma, 500 ng/ml, Cat. No. I-0634) and GolgiPlug™ (BD Cat. No. 555029) for 4 hours at 37°C. The activated cells were harvested and the level of IFN- γ producing cells were detected by immunocytochemistry using a three-step staining procedure that employs a Biotin Goat anti-rat IgG secondary antibody (BD Cat. No. 559286) and a horseradish peroxidase-based detection system (Streptavidin-HRP BD Cat. No. 550946)(Nomarski optics, original magnification 400X).

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 purified format of the XMG1.2 (Cat. No. 559065) antibody can be used to identify and enumerate human IFN- γ producing cells by immunocytochemistry. For optimal indirect immunocytochemical staining, the antibody should be titrated and visualized via a three-step staining procedure. Please see protocol below for a detailed description of the immunocytochemical procedure. The avidin/biotin method is a highly sensitive method, because it employs a mixture of avidin and biotinylated enzyme complexes to increase immunoenzymatic signals. For optimal detection of cytokine producing cells, horseradish peroxidase is the preferred enzyme system.

CYTOKINE IMMUNOCYTOCHEMISTRY PROTOCOL

REAGENTS REQUIRED

1. Fixation Buffer: 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), or BD Pharmingen™ ICC Fixation Buffer (BD Cat. No. 550010)
2. Endogenous Peroxidase Blocking Buffer: DAKO Peroxidase Blocking Reagent (DAKO, Cat. No. S2001).

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.259.0187	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

For country-specific contact information, visit bdbiosciences.com/how_to_order/

Conditions: The information disclosed herein is not to be construed as a recommendation to use the above product in violation of any patents. BD Biosciences will not be held responsible for patent infringement or other violations that may occur with the use of our products. Purchase does not include or carry any right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Becton Dickinson and Company is strictly prohibited.

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale.

BD, BD Logo and all other trademarks are the property of Becton, Dickinson and Company. ©2006 BD



BD

BD Biosciences

3. Endogenous Biotin Blocking Buffer: Biotin/Avidin Blocking Kit (Vector Laboratories, Cat. No. SP-2001).
4. Antibody dilution buffer: BD Pharmingen™ Cytokine ICC Diluent Buffer supplemented with saponin (BD Cat. No. 550009).
5. Microscopic slides: Adhesion Slides (Erie Scientific Company, Cat. No. ER-202B-AD) or for cytopsins, Colorfrost /Plus slides (Fisher, Cat. No. 12-550-17).
6. Detection system: BD Pharmingen™ Streptavidin-horseradish peroxidase (HRP), (Cat. No. 550946) or Anti-Rat Ig HRP Detection Kit (Cat. No. 551013).
7. Mounting medium for short-term storage: Aqua-mount® (Lerner Laboratories, Cat. No. 13800).
8. DAB Substrate Kit (contains 3-3'-Diaminobenzidine tetra hydrochloride), (BD Cat. No. 550880 or Anti-Rat Ig HRP Detection Kit (Cat. No. 551013).

SECONDARY ANTIBODIES

1. Biotin Goat anti-Rat IgG (Cat. No. 559286), or 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 (cytopsins).

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 10⁶ 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 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 Cytokine ICC Diluent Buffer 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 ICC Cytokine 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 10⁶ 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
551013	Anti-Rat Ig HRP Detection Kit	200 tests	(none)
555029	Protein Transport Inhibitor (Containing Brefeldin A)	1.0 ml	(none)
559286	Biotin Polyclonal Goat Anti-Rat IgG	0.5 mg	Polyclonal
559072	Purified Rat IgG1 κ Isotype Control	0.25 mg	R3-34

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharming/en/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 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.(Clone-specific)

Cherwinski HM, Schumacher JH, Brown KD, Mosmann TR. Two types of mouse helper T cell clone. III. Further differences in lymphokine synthesis between Th1 and Th2 clones revealed by RNA hybridization, functionally monospecific bioassays, and monoclonal antibodies. *J Exp Med.* 1987; 166(5):1229-1244. (Clone-specific)

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: Immunocytochemistry (cytospins))

Hsu SM, Raine L, Fanger H. A comparative study of the peroxidase-antiperoxidase method and an avidin-biotin complex method for studying polypeptide hormones with radioimmunoassay antibodies. *Am J Clin Pathol.* 1981; 75(5):734-738.(Methodology: Immunocytochemistry (cytospins))

Sander B, Hoiden I, Andersson U, Moller E, Abrams JS. Similar frequencies and kinetics of cytokine producing cells in murine peripheral blood and spleen. Cytokine detection by immunoassay and intracellular immunostaining. *J Immunol Methods.* 1993; 166(2):201-214.(Clone-specific: Immunocytochemistry (cytospins))