Technical Data Sheet

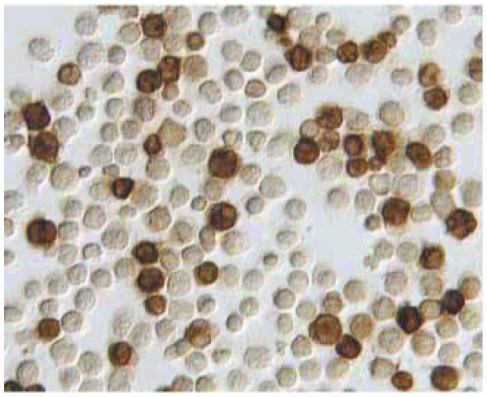
Purified Mouse Anti-Human IFN-γ

Product Information

Material Number:	**550011**
Size:	0.25 mg
Concentration:	0.5 mg/ml
Clone:	B27
Immunogen:	Human E.coli-expressed IFN-γ
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The B27 antibody reacts with human interferon-γ (IFN-γ). The immunogen used to generate the B27 hybridoma was *E.coli*-expressed recombinant human IFN-γ. This is a neutralizing antibody. The use of B27 antibody for epitope mapping of human IFN-γ has been described. The B27 antibody has been reported not to bind to denatured IFN-γ.



***Immunocytochemistry**: PBMC were isolated from human peripheral blood by density gradient cetrifugation and were cultured for 2 days with plate bound anti-human CD3 and soluble anti-human CD28 in the presence of recombinant human IL-2 and recombinant IL-4. The cells were subsequently harvested, washed, and recultured with recombinant human IL-2 and recombinant human IL-4 for an additional 3 days. Finally, the cells were harvested, washed, and stimulated with PMA (Sigma, 5 ng/ml) and ionomycin (Sigma, 500 ng/ml) in the presence of GolgiStop™ (Cat. No. 554724) for 4 hours at 37°C. The activated cells were harvested and the level of IFN-γ producing cells were detected by a three-step staining procedure that employs a Biotin Goat anti-mouse IgG secondary antibody (Cat. No. 550337) and Streptavidin-horseradish peroxidase (HRP), 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 B27 (Cat. No. 550011) antibody can be used to identify and enumerate human IFN-γ producing cells by immunocytochemistry. For optimal indirect immunocytochemical staining, the B27 antibody should be titrated (≤ 1 µg) 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).
3. Endogenous Biotin Blocking Buffer: Biotin/Avidin Blocking Kit (Vector Laboratories, Cat. No. SP-2001).
4. Antibody dilution buffer: BD™ Pharmingen Antibody Diluent for IHC, Cat. No. 559148, supplemented with saponin.

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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. Detection system: BD Pharmingen Streptavidin-horseradish peroxidase (HRP), (Cat. No. 550946).
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)

SECONDARY ANTIBODIES
1. Biotin Goat anti-Mouse IgG (Cat. No. 550337)

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 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) 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 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 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 mounding 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
550010	ICC Fixation Buffer	100 ml	(none)
559148	Antibody Diluent for IHC	125 ml	(none)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
550880	DAB Substrate Kit	500 tests	(none)
550946	Streptavidin HRP	50 ml	(none)
550337	Biotin Goat Anti-Mouse Igs	1.0 ml	Polyclonal
550878	Purified Mouse IgG1 κ Isotype Control	1.0 ml	MOPC-31C

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

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