Technical Data Sheet

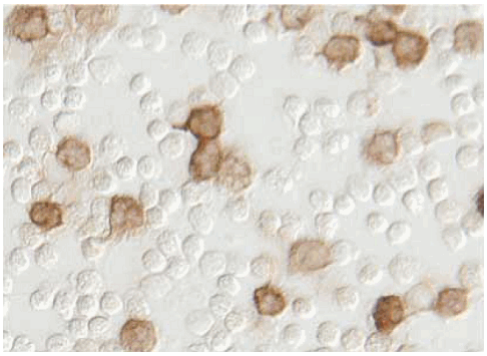
Purified Mouse Anti-Human IL-8

Product Information

Material Number:	**550419**
Size:	0.25 mg
Concentration:	0.5 mg/ml
Clone:	G265-8
Immunogen:	Recombinant Human IL-8
Isotype:	Mouse IgG2b
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The G265-8 antibody reacts with both the 72 and 77 amino acid forms of human interleukin-8 (IL-8). The immunogen used to produce the G265-8 hybridoma was E. coli-expressed recombinant human IL-8.



PBMC were isolated from human peripheral blood by density gradient centrifugation and were cultured overnight at 37°C with LPS (Sigma No. L-8274, 1 µg / ml) in the presence of GolgiStop™ (Cat. No. 554724). *The activated cells were harvested and the level of IL-8 producing cells was detected by immunocytochemistry using a three-step staining procedure that employs a Biotin Goat anti-mouse IgG secondary antibody (Cat. No. 550337) and Streptavdin-horseradish peroxidase (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 G265-8 antibody (Cat. No. 550419) can be used to identify and enumerate human IL-8 producing cells by immunocytochemistry. For optimal indirect immunocytochemical staining, the G265-8 antibody should be titrated (≤ 1 µg) and visualized via a three step staining procedure in combination with Biotin Goat Anti-Mouse IgG and Streptavidin-horseradish peroxidase (HRP). For a detailed description of the immunocytochemical procedure, please see protocol below.

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.
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-Mouse IgG (Cat. No. 550337) or the Anti-Mouse Ig HRP Detection Kit (Cat. No. 551011).
7. Detection system: BD Pharmingen™ Streptavidin-horseradish peroxidase (HRP), (Cat. No. 550946), or Anti-Mouse Ig HRP Detection Kit (Cat. No. 551011).

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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-Mouse Ig HRP Detection Kit.

PROCEDURE FOR IMMUNOCYTOCHEMICAL STAINING OF SINGLE-CELL PREPARATIONS

This procedure describes the immunoenzymatic technique of staining cytokine 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, 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 ìl 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 Antibody Diluent for IHC 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 Antibody Diluent for IHC 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
550337	Biotin Goat Anti-Mouse Ig (Multiple Adsorption)	1.0 ml	Polyclonal
550946	Streptavidin HRP	50 ml	(none)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
550010	ICC Fixation Buffer	100 ml	(none)
551011	Anti-Mouse Ig HRP Detection Kit	200 tests	(none)
550880	DAB Substrate Kit	500 tests	(none)
559148	Antibody Diluent for IHC	125 ml	(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. 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

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)

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)

Matsushima K, Oppenheim JJ. Interleukin 8 and MCAF: novel inflammatory cytokines inducible by IL 1 and TNF. *Cytokine.* 1989; 1(1):2-13.(Biology)