Technical Data Sheet

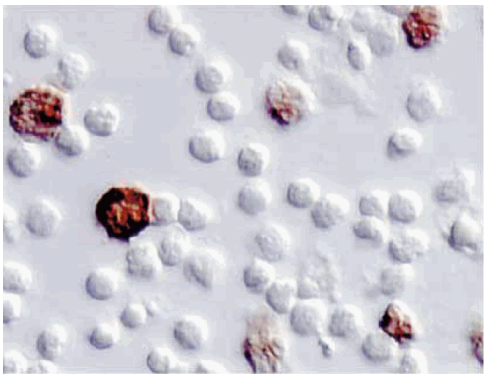
Purified Rat Anti-Human IL-6

Product Information

Material Number:	**559068**
Size:	0.25 mg
Concentration:	0.5 mg/ml
Clone:	MQ2-6A3
Immunogen:	Recombinant human IL-6
Isotype:	Rat IgG2a, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The MQ2-6A3 antibody reacts with human interleukin-6 (IL-6). The immunogen used to generate the MQ2-6A3 hybridoma was recombinant human IL-6. This is a neutralizing antibody.



PBMC were isolated from human peripheral blood by density gradient centrifugation and were cultured with PMA (Sigma, Cat. No. P-8139, 5 ng/ml) and ionomycin (Sigma, Cat. #I-0634, 500 ng/ml) in the presence of GolgiStop™ (Cat. No. 554724) overnight at 37°C. *The activated cells were harvested and the presence of IL-6 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. To demonstrate specificity of staining the binding of the MQ2-6A3 (Cat. No. 559068) antibody was blocked by the preincubation of the purified antibody with excess recombinant human IL-6 protein (Cat. No. 550071; data not shown). (Nomarski optics, original magnification 400X).*

Preparation and Storage

Store undiluted at 4°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Application Notes

Application

Immunocytochemistry (cytospins)	Routinely Tested
Intracellular block/flow cytometry	Tested During Development

Recommended Assay Procedure:

Immunocytochemistry: The ICC format of the purified MQ2-6A3 (Cat. No. 559068) antibody can be used to identify and enumerate human IL-6 producing cells by immunocytochemistry. For optimal indirect immunocytochemical staining, the MQ2-6A3 antibody should be titrated (≤ 1 µg) and visualized via a three-step staining procedure in combination with Biotin Goat Anti-Rat IgG (Cat. No. 559286) and Streptavidin-HRP (Cat. No. 550946).

* The Avidin/ Biotin method is a highly sensitive method for the detection of cytokines associated with single cells. It employs a mixture of avidin and biotinylated enzyme complexes in order to increase immunoenzymatic signals. For optimal detection of cytokine producing cells, Pharmingen recommends horseradish peroxidase as the preferred enzyme system.

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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: Pharmingen's 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. Streptavidin - HRP (Cat. No. 550946) or or the Anti-Mouse Ig HRP Detection Kit (Cat. No. 551011).
7. Mounting medium for short-term storage: Aqua-mount® (Lerner Laboratories, Cat. No. 13800).
8. DAB Substrate Kit (Cat. No. 550880) or the Anti-Rat Ig HRP Detection Kit (Cat. No. 551013).\

SECONDARY ANTIBODY

1. Biotin Goat anti-Rat IgG (Cat. No. 559286), or the Anti-Rat Ig HRP Detection Kit (Cat. No. 551013), containing all detection reagents needed.

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 x 10^6 to 5 x 10^6 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 IHC Diluent (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 for 30 min at RT.
19. Wash 2X in PBS with 5 min incubations.
20. Apply 20 µl of Streptavidin . HRP (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 3-3'-Diaminobenzidine tetra hydrochloride (DAB), (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
550946	Streptavidin HRP	50 ml	(none)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
550880	DAB Substrate Kit	500 tests	(none)
559286	Biotin Goat Anti-Rat Ig	0.5 mg	Polyclonal
550010	ICC Fixation Buffer	100 ml	(none)
559148	Antibody Diluent for IHC	125 ml	(none)
551013	Anti-Rat Ig HRP Detection Kit	200 tests	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. 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.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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