Technical Data Sheet

Purified Rat Anti-Human IL-10

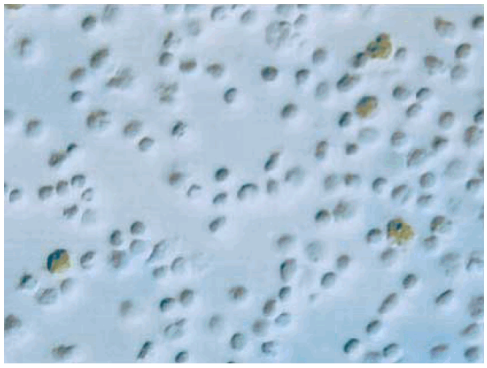
Product Information

Material Number:	**559076**
Size:	0.25 mg
Concentration:	0.5 mg/ml
Clone:	JES3-12G8
Immunogen:	Human IL-10
Isotype:	Rat IgG2a
Reactivity:	QC Testing: Human Tested in development: Viral
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The JES3-12G8 antibody reacts with human interleukin-10 (IL-10) and viral IL-10. The immunogen used to generate the JES3-12G8 hybridoma was human IL-10. 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.



***Human IL-10 Staining**. PBMC were isolated from human peripheral blood by density gradient centrifugation 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 human 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) and ionomycin (Sigma) in the presence of GolgiStop™ (Cat. No. 554724) for 4 hours at 37°C. The activated cells were harvested and the presence of IL-10 producing cells was detected by immunocytochemistry using a three-step staining procedure that employs a biotin goat anti-rat IgG secondary antibody and a horseradish peroxidase-based detection system (see protocol below) (Nomarski optics, original magnification 400 X). To demonstrate specificity of staining the binding of JES3-12G8 (Cat. No. 559076) antibody was blocked by the preincubation of the purified antibody with excess recombinant human IL-10 protein (Cat. No. 554611; 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 JES3-12G8 (Cat. No. 559076) antibody can be used to identify and enumerate human IL-10 producing cells by immunocytochemistry. For optimal indirect immunocytochemical staining, the JES3-12G8 antibody should be titrated and visualized via a three-step staining procedure in combination with biotin goat anti-Rat IgG and an avidin/streptavidin horseradish peroxidase detection system. A detailed protocol for the cytokine immunocytochemical procedure follows.

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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: 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. 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 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 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 Antibody Diluent for IHC, 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
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)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
559073	Purified Rat IgG2a κ Isotype Control (ICC)	0.25 mg	R35-95

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 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: ELISA)

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