Technical Data Sheet

Purified Rat IgG1, κ Isotype Control

Product Information

Material Number:	**559072**
Alternate Name:	IgG1, kappa; Ighg1, Igkc
Size:	0.25 mg
Concentration:	0.5 mg/ml
Clone:	R3-34
Immunogen:	Mouse immunoglobulin
Isotype:	Rat IgG1, κ
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

Following immunization of a rat with mouse immunoglobulin (Ig), the Ig from the R3-34 hybridoma was identified as a non-reactive clone. The R3-34 immunoglobulin was selected as an Ig isotype control following screening for low background staining on a variety of mouse and human cells and tissues.

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

Isotype control	Routinely Tested
ELISA Standard	Routinely Tested
Flow cytometry	Routinely Tested
Immunocytochemistry (cytospins)	Tested During Development
Immunohistochemistry-formalin (antigen retrieval required)	Tested During Development
Immunohistochemistry-frozen	Tested During Development
Immunohistochemistry-zinc-fixed	Tested During Development

Recommended Assay Procedure:

Immunocytochemistry: Investigator are advised that this pure is not routinely tested using the Immunocytochemisty (Cytospins) application. This material is an immunoglobulin isotype control for Rat IgG1 and can be used to determine the level of non-specific background staining in an indirect cytokine immunocytochemical assay. Purified R3-34 antibody should be utilized at the same concentration as the primary specific antibody and visualized under the same conditions via a three step staining procedure in combination with Biotin Goat Anti-Rat IgG (Cat. No. 559286) and an avidin or streptavidin-horseradish peroxidase (Cat. No. 550946) detection system.

CYTOKINE IMMUNOCYTOCHEMISTRY PROTOCOL

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).

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™ 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 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).

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SECONDARY ANTIBODIES

1. Biotin Goat anti-Rat IgG (Cat. No. 559286) or Anti-Rat Ig HRP Detection Kit (Cat. No. 551013).
2. Biotin Goat anti-Mouse IgG (Cat. No. 550337) or Anti-Mouse Ig HRP Detection Kit (Cat. No. 551011).

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 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 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
559286	Biotin Goat Anti-Rat Ig	0.5 mg	Polyclonal
550337	Biotin Goat Anti-Mouse Ig (Multiple Adsorption)	0.25 mg	Polyclonal
550946	Streptavidin HRP	50 mL	(none)
551013	Anti-Rat Ig HRP Detection Kit	200 Tests	(none)
551011	Anti-Mouse Ig HRP Detection Kit	200 Tests	(none)
550880	DAB Substrate Kit	500 Tests	(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.
4. An isotype control should be used at the same concentration as the antibody of interest.

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. (Clone-specific: 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. (Clone-specific: Immunocytochemistry (cytospins))

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