Technical Data Sheet

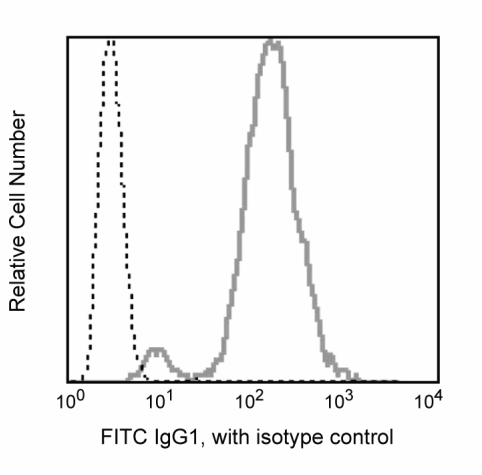
FITC Rat Anti-Mouse IgG1

Product Information

Material Number:	**562026**
Alternate Name:	Ighg1; Immunoglobulin heavy constant gamma 1; Igh-4
Size:	25 µg
Concentration:	0.5 mg/ml
Clone:	A85-1
Immunogen:	Pooled Mouse IgG1
Isotype:	Rat (LOU) IgG1, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing protein stabilizer and ≤0.09% sodium azide.

Description

The A85-1 antibody reacts specifically with mouse IgG1 of Igh-Ca and Igh-Cb haplotypes. It does not react with other Ig isotypes. Detection of surface immunoglobulin on B lymphoma cells has been demonstrated with the A85-1 monoclonal antibody. A suspension of pooled mouse IgG1 was used as the source of immunogen.



Detection of intracellular mouse IgG1 in an antibody-secreting hybridoma cell line. *Cells were fixed, permeabilized, and stained according to the method described below using FITC Rat anti-Mouse IgG1 mAb (solid line) or FITC Rat IgG1, κ Isotype Control(dotted line, Cat. No. 554684). Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.
The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with FITC under optimum conditions, and unreacted FITC was removed.

Application Notes

Application

Flow cytometry	Routinely Tested
Intracellular staining (flow cytometry)	Tested During Development

Recommended Assay Procedure:

FITC-conjugated A85-1 antibody may be used as a primary or secondary reagent in immunofluorescent staining.

IMMUNOFLUORESCENT STAINING OF INTRACELLULAR IMMUNOGLOBULIN (Ig) PROTOCOL

1. Prepare a single-cell suspension and determine cell number.
2. Suspend cells in BD Pharmingen™ Stain Buffer with FBS (Cat. No. 554656) at 2 x 10^7 cells/ml and transfer to U-bottom microwell plates in 50 µl/well for immunofluorescent staining.
3. Block Fcγ receptors by adding 0.2 µg of purified 2.4G2 antibody (Mouse BD Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2) (Cat. No. 553141/553142) in 50 µl of staining buffer to each well.
4. Incubate 5 minutes on ice.

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5. Add 200 µl of staining buffer/well and resuspend cells. Centrifuge at 250 x g for 5 minutes and aspirate supernatant.
6. Block surface Ig with purified A85-1 mAb (Cat. No. 553440) by adding 1.0 µg per sample in 50 µl of staining buffer/well.
7. Incubate 15 minutes on ice.
8. Wash 2x as described in Step 5.
9. Resuspend cells in 100 µl of BD Cytofix/Cytoperm™ intracellular staining buffer (BD Cytofix/Cytoperm™ Kit, Cat. No. 554714) per well.
10. Incubate 30 minutes at room temperature.
11. Wash 2x with 200 µl of 1x Perm/Wash buffer (provided in the BD Cytofix/Cytoperm Kit) per well. Centrifuge at 250 x g for 5 minutes and aspirate supernatant between washes.
12. Stain intracellular Ig by adding ≤1 µg of FITC-conjugated A85-1 mAb in 50 µl of 1 x Perm/Wash buffer/well.
    Note: Other antibodies recommended for staining of intracellular markers may be added during this step as described in Step 12.
13. Incubate for 30 minutes at room temperature.
14. Wash 2x as described in Step 11.
15. Resuspend and transfer samples in 100 µl of staining buffer to tubes appropriate for analysis with a flow cytometer. Bring volume in each tube to 400 µl with staining buffer.
16. Analyze samples on a flow cytometer.

Note: Surface markers may be stained during this step as described in the "Immunofluorescent Staining of Mouse and Rat Leukocytes for Flow Cytometry" in the Technical Protocols section of our website at http://www.bdbiosciences.com/support/resources/flowcytometry/index.jsp

Suggested Companion Products

Catalog Number	Name	Size	Clone
554714	BD Cytofix/Cytoperm™ Fixation/Permeablization Kit	250 tests	(none)
554684	FITC Rat IgG1, κ Isotype Control	0.1 mg	R3-34
554656	Stain Buffer (FBS)	500 ml	(none)
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2

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. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
5. An isotype control should be used at the same concentration as the antibody of interest.