Technical Data Sheet

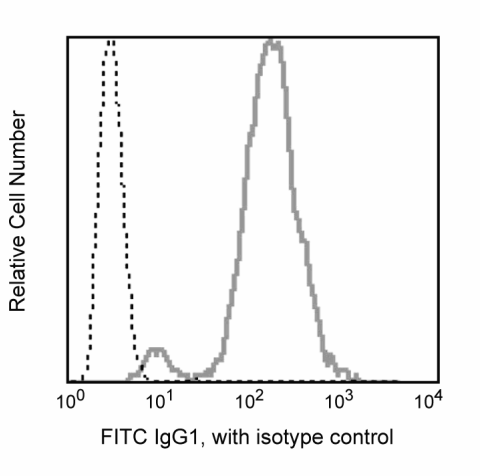
FITC Rat Anti-Mouse IgG1

Product Information

Material Number:	**553443**
Alternate Name:	Ighg1; Immunoglobulin heavy constant gamma 1; Igh-4
Size:	0.5 mg
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.



Flow cytometric analysis of IgG1 expression in an antibody-secreting hybridoma cell line. *Hybridoma cell line was stained with either FITC Rat Anti-Mouse IgG1 (Cat. No. 553443/562026; solid line histogram), or FITC Rat IgG1, κ Isotype Control (Cat. No. 554684; dashed line histogram). The fluorescence histograms were derived from gated events with the forward and side light-scattering characteristics of viable hybridoma cells. Flow cytometry was performed on a BD FACSCalibur™.*

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:

BD Pharmingen™ FITC Rat Anti-Mouse IgG1 antibody may be used as a primary or secondary reagent in immunofluorescent staining.

BD Biosciences

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IMMUNOFLUORESCENT STAINING OF INTRACELLULAR IMMUNOGLOBULIN (Ig) PROTOCOL

1. Prepare a single-cell suspension and determine cell number.
2. Suspend cells in staining buffer (PBS + 2% FBS + 0.1% Sodium Azide) at 2 x 10^7 cells/ml and transfer to U-bottom microwell plates in 50 µl/well for immunofluorescent staining.

Note: The BD Pharmingen™ Stain Buffer with FBS (Cat. No. 554656) is effective for use as a staining buffer in this protocol.

3. Block Fcγ receptors by adding 0.2 µg of Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™) (Cat. No. 553141/553142) in 50 µl of staining buffer to each well.
4. Incubate 5 minutes on ice.
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 Rat Anti-Mouse IgG1(Cat. No. 553440) by adding 1.0 µg per sample in 50 µl of staining buffer/well.

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 Protocols section of our website under "Multicolor Flow" at http://www.bdbiosciences.com/us/s/resources.

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 BD Pharmingen™ FITC Rat Anti-Mouse IgG1(Cat. No. 553443/562026) 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.

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
553142	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.5 mg	2.4G2
553440	Purified Rat Anti-Mouse IgG1	0.5 mg	A85-1
562026	FITC Rat Anti-Mouse IgG1	25 µg	A85-1
554657	Stain Buffer (BSA)	500 mL	(none)

Product Notices

1. An isotype control should be used at the same concentration as the antibody of interest.
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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
5. Since applications vary, each investigator should titrate the reagent to obtain optimal results.