Technical Data Sheet

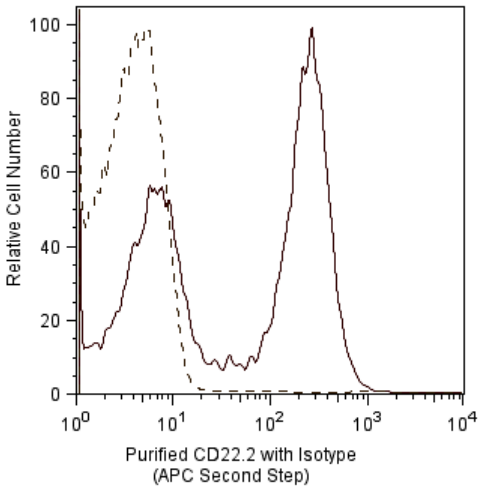
APC Rat anti-Mouse IgG1

Product Information

Material Number:	**560089**
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	A85-1
Immunogen:	Pooled Mouse IgG1
Isotype:	Rat (LOU) IgG1, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤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 A85-1 mAb. A suspension of pooled mouse IgG1 was used as the source of immunogen.



Flow cytometric analysis of APC-conjugated anti-mouse IgG1 second step. *Freshly isolated BALB/c splenocytes were incubated with the primary purified antibody for mouse CD22.2 (clone Cy34.1, solid line) followed by APC-conjugated anti-MouseIgG1 as second step (Clone A85-1, Cat.No. 560089) and compared to an APC-conjugated isotype control (Cat. No. 554686, dashed line). Flow cytometry was performed on a BD FACSCalibur™ System and the histograms were derived from the gated events based on light scattering characteristics of viable splenocytes.*

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 to APC under optimum conditions, and unconjugated antibody and free APC were removed.

Application Notes

Application

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

Recommended Assay Procedure:

APC-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 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.

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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.
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.

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/protocols/mouse_rat_leukocytes.jsp

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 APC-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.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554686	APC Rat IgG1, κ Isotype Control	0.1 mg	R3-34
554714	BD Cytofix/Cytoperm™ Fixation/Permeablization Kit	250 tests	(none)
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
554656	Stain Buffer (FBS)	500 ml	(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. This APC-conjugated reagent can be used in any flow cytometer equipped with a dye, HeNe, or red diode laser.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
5. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.