Technical Data Sheet

FITC Mouse Anti-Rat IgG1

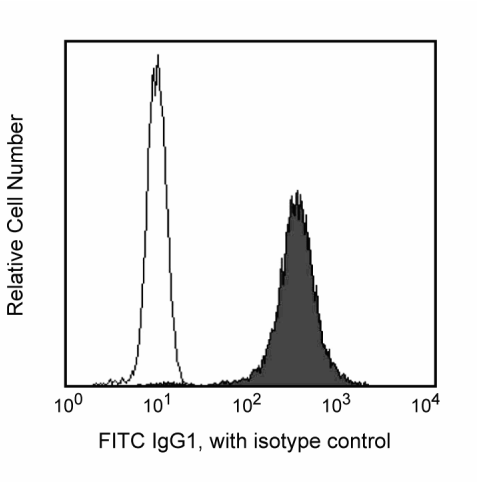
Product Information

Material Number:	**553892**
Size:	0.5 mg
Concentration:	0.5 mg/ml
Clone:	RG11/39.4
Immunogen:	Pooled rat IgG1
Isotype:	Mouse (SJL) IgG2a, κ
Reactivity:	QC Testing: Rat
Storage Buffer:	Aqueous buffered solution containing protein stabilizer and ≤0.09% sodium azide.

Description

The RG11/39.4 antibody reacts specifically with the Fc region of rat IgG1. It does not react with other Ig isotypes.

This antibody is routinely tested by flow cytometric analysis. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.



Detection of intracellular rat IgG1 in an antibody-secreting hybridoma cell line. *Cells were fixed, permeabilized, and stained according to the method described below using FITC-conjugated RG11/39.4 mAb (filled histogram) or the matched isotype control, FITC-conjugated 27-35 mAb (open histogram, Cat. No. 555057). Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*

Preparation and Storage

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.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

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

Recommended Assay Procedure:

RG11/39.4 antibody is effective for detection of cell-surface or intracellular Ig by immunofluorescent staining with flow cytometric analysis. FITC-conjugated RG11/39.4 mAb 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 × 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 × g for 5 minutes and aspirate supernatant.
6. Block surface Ig with purified RG11/39.4 mAb (Cat. No. 553889) 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 web site at http://www.bdbiosciences.com/pharmingen/protocols/Mouse_and_Rat_Leukocytes.shtml

7. Incubate 15 minutes on ice.
8. Wash 2× 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 2× with 200 µl of 1× BD Perm/Wash™ buffer (provided in the BD Cytofix/Cytoperm Kit) per well. Centrifuge at 250 × g for 5 minutes and aspirate supernatant between washes.
12. Stain intracellular Ig by adding ≤ 1 µg of FITC-conjugated RG11/39.4 mAb in 50 µl of 1 × BD 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 2× 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
554656	Stain Buffer (FBS)	500 ml	(none)
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
553889	Purified Mouse Anti-Rat IgG1	0.5 mg	RG11/39.4
554714	BD Cytofix/Cytoperm™ Fixation/Permeablization Kit	250 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.

References

Springer TA, Bhattacharya A, Cardoza JT, Sanchez-Madrid F. Monoclonal antibodies specific for rat IgG1, IgG2a, and IgG2b subclasses, and kappa chain monotypic and allotypic determinants: reagents for use with rat monoclonal antibodies. *Hybridoma.* 1982; 1(3):257-273.(Immunogen)