Technical Data Sheet

FITC Rat Anti-Mouse IgG2b

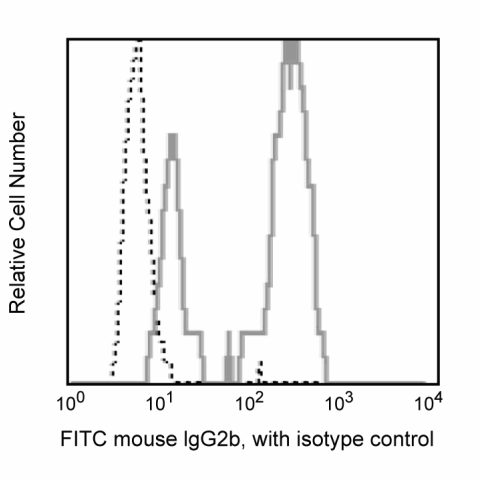
Product Information

Material Number:	**553395**
Size:	0.5 mg
Concentration:	0.5 mg/ml
Clone:	R12-3
Immunogen:	Pooled Mouse Ig
Isotype:	Rat (LOU) IgG2a, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The R12-3 antibody recognizes an epitope in the CH3 domain of mouse IgG2b of *Igh-C[a]* and *Igh-C[b]* haplotypes. It does not react with other Ig isotypes. Detection of surface immunoglobulin on B lymphoma cells has been demonstrated with R12-3 mAb.

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 mouse IgG2b in an antibody-secreting hybridoma cell line. *Cells were fixed, permeabilized, and stained according to the method described below using FITC R12-3 mAb (solid line, Cat. No. 553395) or the matched isotype control, FITC R35-95 mAb (dotted line, Cat. No. 554688). 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)	Routinely Tested

Recommended Assay Procedure:

Immunostaining and flow cytometry: FITC-conjugated R12-3 mAb may be used as a primary or secondary reagent in immunofluorescent staining. For detection of intracytoplasmic IgG2b, please refer to the following protocol.

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1. Prepare a single-cell suspension and determine cell number.
2. Suspend cells in staining buffer (PBS + 2% FBS + 0.1% Sodium Azide, Stain buffer, Cat. No. 554656) at 2 × 10e7 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.
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 R12-3 mAb (Cat. no. 553392) 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/pharmingen/protocols/Mouse_and_Rat_Leukocytes.shtml
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 R12-3 mAb in 50 µl of 1X 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
554688	FITC Rat IgG2a, κ Isotype Control	0.1 mg	R35-95
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
553392	Purified Rat Anti-Mouse IgG2b	0.5 mg	R12-3

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.

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