Technical Data Sheet

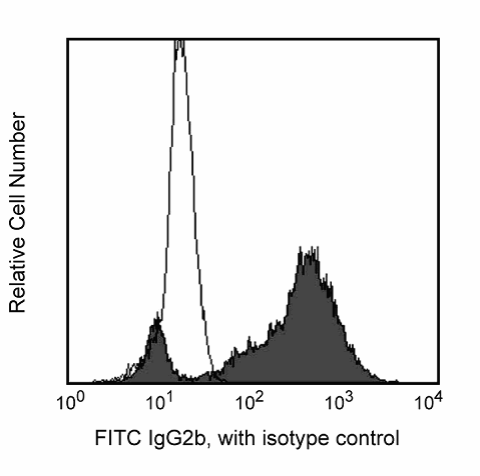
FITC Mouse Anti-Rat IgG2b

Product Information

Material Number:	**553884**
Size:	0.5 mg
Concentration:	0.5 mg/ml
Clone:	G15-337
Immunogen:	Pooled rat Ig
Isotype:	Mouse IgG2b, κ
Reactivity:	QC Testing: Rat
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The G15-337 antibody reacts specifically with rat IgG2b. It does not react with other Ig isotypes. A suspension of pooled rat Ig was used as the source of immunogen.



Detection of intracellular rat IgG2b in an antibody-secreting hybridoma cell line. *Cells were fixed, permeabilized, and stained according to the method described below using FITC-conjugated G15-337 mAb (shaded histogram) or the matched isotype control, FITC-conjugated 27-35 mAb (empty 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)	Routinely Tested

Recommended Assay Procedure:
G15-337 antibody is effective for detection of cell-surface or intracellular Ig by immunofluorescent staining with flow cytometric analysis.
FITC-conjugated G15-337 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 x 10e7 cells/ml and transfer to U-bottom microwell plates in 50µl/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 G15-337 mAb (Cat. No. 553882) 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 (see 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 G15-337 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
555057	FITC Mouse IgG2b, κ Isotype Control	0.1 mg	27-35
554656	Stain Buffer (FBS)	500 ml	(none)
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
553882	Purified Mouse Anti-Rat IgG2b	0.5 mg	G15-337
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.