Technical Data Sheet

FITC Rat Anti-Mouse IgM

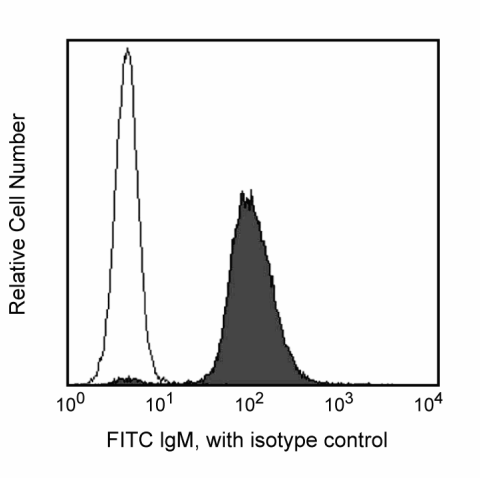
Product Information

Material Number:	**553437**
Size:	0.5 mg
Concentration:	0.5 mg/ml
Clone:	II/41
Immunogen:	Not reported
Isotype:	Rat IgG2a, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The II/41 clone has been reported to react specifically with mouse IgM of Igh-C[a] and Igh-C[b] haplotypes. It has been reported not to react with other Ig isotypes. In addition, the II/41 clone has been reported not to stimulate B-cell proliferation.

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 IgM in an antibody-secreting hybridoma cell line. *Cells were fixed, permeabilized, and stained according to the method described in the recommended assay procedure using FITC-conjugated rat anti-mouse IgM (clone II/41) (filled histogram, Cat. No. 553437) or the matched isotype control, FITC rat IgG2a (clone R35-95) (open histogram, Cat. No. 554688). Flow cytometry was performed on a BD FACSCalibur™ instrument.*

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

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Recommended Assay Procedure:

Immunofluorescent Staining of Mouse Intracytoplasmic/Intracellular IgM:

1. Prepare a single-cell suspension and determine cell number.
2. Suspend cells at 2×10e7 cells/ml in BD Pharmingen™ Stain Buffer (Cat. No. 554656) [or alternatively, prepare a staining buffer made up in PBS + 2% FBS + 0.1% sodium azide] and transfer to U-bottom microwell plates in 50 µl/well for immunofluorescent staining.
3. Block Fcγ receptors by adding 0.2 µg of BD Fc Block™ (Cat. no. 553141) 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 cell surface IgM with purified rat anti-mouse IgM (clone II/41) (Cat. No. 553435) 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™ solution (Cat. No. 554714) per well.
10. Incubate 30 minutes at room temperature.
11. Wash 2× with 200 µl of 1× BD Perm/Wash™ buffer (Cat.No. 554714) per well. Centrifuge at 250×g for 5 minutes and aspirate supernatant between washes.
12. Stain for intracellular IgM by adding ≤ 1 µg of FITC rat anti-mouse IgM (clone II/41) antibody 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
554714	BD Cytofix/Cytoperm Fixation/Permeablization Kit	250 tests	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
554688	FITC Rat IgG2a, κ Isotype Control	0.1 mg	R35-95
553435	Purified Rat Anti-Mouse IgM	0.5 mg	II/41

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

Laszlo G, Hathcock KS, Dickler HB, Hodes RJ. Characterization of a novel cell-surface molecule expressed on subpopulations of activated T and B cells. *J Immunol.* 1993; 150(12):5252-5262.(Biology)