Technical Data Sheet **Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)**

Product Information	
Material Number:	553142
Alternate Name:	Fcy III/II Receptor
Size:	0.5 mg
Concentration:	0.5 mg/ml
Clone:	2.4G2
Immunogen:	Mouse BALB/c Macrophage J774 Cell Line
Isotype:	Rat IgG2b ĸ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The 2.4G2 antibody reacts specifically with a common nonpolymorphic epitope on the extracellular domains of the mouse FcyIII and FcyII receptors. It has also been reported to bind the FcyI receptor (CD64) via its Fc domain. 2.4G2 mAb blocks non-antigen-specific binding of immunoglobulins to the FcyIII and FcyII, and possibly FcyI, receptors *in vitro* and *in vivo*. CD16 and/or CD32 are expressed on natural killer cells, monocytes, macrophages, dendritic cells (at low levels), Kupffer cells, granulocytes, mast cells, B lymphocytes, immature thymocytes, and some activated mature T lymphocytes.

This antibody is routinely tested by flow cytometric analysis.



of CD16/CD32 on mouse spleen cells and demonstration of FCyR-mediated non-specific staining. Left: BALB/c splenocytes were simultaneously stained with PE-conjugated anti-mouse CD3e mAb 145-2C11 (Cat. No. 553063/553064) and purified 2.4G2 mAb. The staining by 2.4G2 antibody was detected with FITC-conjugated mouse anti-rat Ig, ĸ chain mAb MRK-1 (Cat. No. 553872). Right: BALB/c splenocytes were stained with FITC-conjugated rat anti-mouse CD90.2 (Thy-1.2) mAb 53-2.1 (Cat. No. 553003/553004) in the presence of purified 2.4G2 mAb (filled histogram) and without 2.4G2 mAb (open histogram). Flow cytometry was performed on a BD FACScan™ flow cytometry system.

Two color analysis of the expression

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography. Store undiluted at 4° C.

Application Notes

Application

Blocking	Routinely Tested
Flow cytometry	Routinely Tested
Immunohistochemistry-frozen	Tested During Development
Immunoprecipitation	Reported

Recommended Assay Procedure:

To specifically stain cells bearing $Fc\gamma II$ and $Fc\gamma III$ receptors for flow cytometric analysis: Incubate cell suspension with this antibody ($\leq 1 \mu g$ /million cells) followed by an appropriate fluorochrome-conjugated second-step reagent.

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To reduce Fc receptor-mediated binding by antibodies of interest or Fc receptor-mediated binding by PE-CY5 tandem dye conjugates to FcyII and FcyIII receptor-bearing mouse cells for flow cytometric analysis:

1. Preincubate cell suspension with Mouse BD Fc BlockTM purified anti-mouse CD16/CD32 mAb 2.4G2 (eg, $\leq 1 \mu g/million$ cells in 100 μ l) at 4°C for 5 minutes.

2. Add antibody of interest directly to preincubated cells in the presence of Mouse BD Fc BlockTM (ie, Mouse BD Fc BlockTM need not be washed off before staining cells).

3. If anti-Ig second-step is necessary, a reagent must be chosen which will not bind to Mouse BD Fc BlockTM (eg, rat IgG_{2b}, κ).

For additional information on using Mouse BD Fc BlockTM, refer to our website protocol at http://www.bdbiosciences.com/pharmingen/protocols/Immunophenotyping.shtml

Product Notices

- 1. 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.
- 2. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
- 3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Araujo-Jorge T, Rivera MT, el Bouhdidi A, Daeron M, Carlier Y. An Fc gamma RII-, Fc gamma RII-specific monoclonal antibody (2.4G2) decreases acute Trypanosoma cruzi infection in mice. *Infect Immun.* 1993; 61(11):4925-4928.(Clone-specific: Blocking)

Benhamou M, Bonnerot C, Fridman WH, Daeron M. Molecular heterogeneity of murine mast cell Fc gamma receptors. *J Immunol.* 1990; 144(8):3071-3077. (Clone-specific: Immunoprecipitation)

Jensen WA, Marschner S, Ott VL, Cambier JC. FcgammaRIIB-mediated inhibition of T-cell receptor signal transduction involves the phosphorylation of SH2-containing inositol 5-phosphatase (SHIP), dephosphorylation of the linker of activated T-cells (LAT) and inhibition of calcium mobilization. *Biochem Soc Trans.* 2001; 29(6):840-846. (Clone-specific: Blocking)

Kaji K, Takeshita S, Miyake K, Takai T, Kudo A. Functional association of CD9 with the Fc gamma receptors in macrophages. *J Immunol.* 2001; 166(5):3256-3265.(Clone-specific: (Co)-stimulation)

Katz HR, Arm JP, Benson AC, Austen KF. Maturation-related changes in the expression of Fc gamma RII and Fc gamma RIII on mouse mast cells derived in vitro and in vivo. J Immunol. 1990; 145(10):3412-3417. (Clone-specific: Immunoprecipitation)

Kurlander RJ, Ellison DM, Hall J. The blockade of Fc receptor-mediated clearance of immune complexes in vivo by a monoclonal antibody (2.4G2) directed against Fc receptors on murine leukocytes. J Immunol. 1984; 133(2):855-862. (Clone-specific: Blocking)

Latour S, Bonnerot C, Fridman WH, Daeron M. Induction of tumor necrosis factor-alpha production by mast cells via Fc gamma R. Role of the Fc gamma RIII gamma subunit. *J Immunol.* 1992; 149(6):2155-2162.(Clone-specific: (Co)-stimulation)

Lewis VA, Koch T, Plutner H, Mellman I. A complementary DNA clone for a macrophage-lymphocyte Fc receptor. *Nature*. 1986; 324(6095):372-375. (Clone-specific)

Maeda K, Burton GF, Padgett DA, et al. Murine follicular dendritic cells and low affinity Fc receptors for IgE (Fc epsilon RII). J Immunol. 1992; 148(8):2340-2347.(Clone-specific: Immunohistochemistry)

Mellman IS, Unkeless JC. Purificaton of a functional mouse Fc receptor through the use of a monoclonal antibody. J Exp Med. 1980;

152(4):1048-1069.(Clone-specific: Immunoprecipitation)

Perussia B, Tutt MM, Qiu WQ, et al. Murine natural killer cells express functional Fc gamma receptor II encoded by the Fc gamma R alpha gene. *J Exp Med.* 1989; 170(1):73-86.(Clone-specific)

Ravetch JV, Luster AD, Weinshank R, et al. Structural heterogeneity and functional domains of murine immunoglobulin G Fc receptors. *Science*. 1986; 234(4777):718-725. (Clone-specific)

Rodewald HR, Awad K, Moingeon P, et al. Fc gamma RII/III and CD2 expression mark distinct subpopulations of immature CD4-CD8- murine thymocytes: in vivo developmental kinetics and T cell receptor beta chain rearrangement status. *J Exp Med.* 1993; 177(4):1079-1092.(Clone-specific: Immunoprecipitation) Rodewald HR, Moingeon P, Lucich JL, Dosiou C, Lopez P, Reinherz EL. A population of early fetal thymocytes expressing Fc gamma RII/III contains precursors of T lymphocytes and natural killer cells. *Cell.* 1992; 69(1):139-150.(Clone-specific: Immunoprecipitation)

Takezawa R, Watanabe Y, Akaike T. Direct evidence of macrophage differentiation from bone marrow cells in the liver: a possible origin of Kupffer cells. J Biochem (Tokyo). 1995; 118(6):1175-1183. (Clone-specific)

Titus JA, Finkelman FD, Stephany DA, Jones JF, Segal DM. Quantitative analysis of Fc gamma receptors on murine spleen cell populations by using dual parameter flow cytometry. *J Immunol.* 1984; 133(2):556-561.(Clone-specific)

Unkeless JC. Characterization of a monoclonal antibody directed against mouse macrophage and lymphocyte Fc receptors. J Exp Med. 1979; 150(3):580-596.(Immunogen)

Vremec D, Zorbas M, Scollay R, et al. The surface phenotype of dendritic cells purified from mouse thymus and spleen: investigation of the CD8 expression by a subpopulation of dendritic cells. J Exp Med. 1992; 176(1):47-58. (Clone-specific)

Witmer MD, Steinman RM. The anatomy of peripheral lymphoid organs with emphasis on accessory cells: light-microscopic immunocytochemical studies of mouse spleen, lymph node, and Peyer's patch. Am J Anat. 1984; 170(3):465-481. (Clone-specific: Immunohistochemistry)