Technical Data Sheet

BV421 Rat Anti-Mouse CD40

Product Information

| Material Number: |
|-----------------------|
| Alternate Name: |
| Size: |
| Concentration: |
| Clone: |
| Immunogen: |
| Isotype: |
| Reactivity: |
| Storage Buffer: |

562846

Bp50; Tnfrsf5; TNR5; TRAP; CD40L receptor; GP39; HIGM1; IMD3; T-BAM 50 μg 0.2 mg/ml 3/23 Mouse CD40 Recombinant Protein Rat (LOU) IgG2a, κ QC Testing: Mouse Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The 3/23 clone monoclonal antibody specifically binds to CD40, a 40-50 kDa glycoprotein expressed on B lymphocytes and other antigen-presenting cells. CD40 has been reported to be transiently expressed on activated CD4+ and CD8+ T cells and in some mouse strains, the 3/23 mAb has been reported to react with 5-10% of T lymphocytes in adult mouse, but not neonatal, spleen. CD40 plays a key role in B-cell growth and differentiation where interactions of CD40 with its ligand, CD154, are involved in the initiation, effector, and memory stages of cell-mediated immune responses. In addition, CD40 has been reported to be involved with the triggering of NK cells and NK-T cells. Ligation of CD40 with the 3/23 antibody has been reported to induce splenic B cells to express the costimulatory molecule CD86 (B7-2). In addition, although the 3/23 antibody by itself is a weak B-cell mitogen, it has been reported to synergize markedly with mitogenic anti-IgD mAb or IL-4 to promote B-cell proliferation.

The antibody was conjugated to BD HorizonTM BV421 which is part of the BD HorizonTM Brilliant VioletTM family of dyes. With an Ex Max of 407-nm and Em Max at 421-nm, BD HorizonTM BV421 can be excited by the violet laser and detected in the standard Pacific BlueTM filter set (eg, 450/50-nm filter). BD HorizonTM BV421 conjugates are very bright, often exhibiting a 10 fold improvement in brightness compared to Pacific BlueTM conjugates.



Two-color flow cytometric analysis of CD40 expression on mouse splenocytes. Mouse splenic leucocytes were stained with FITC Rat Anti-Mouse CD4 (Cat. No. 553047/553046/561835) and FITC Rat Anti-Mouse CD8a (Cat. No. 553031/553030/561966), and either BD Horizon™ BV421 Rat IgC2a, k lsotype Control (Cat. No. 562602; Left Panel) or BD Horizon™ BV421 Rat Anti-Mouse CD40 antibody (Cat. No. 562846; Right Panel). Two-color flow cytometric dot plots show the correlated expression of CD4 + CD8 versus CD40 (or Ig Isotype Control staining) for gated events with the forward and side light-scatter characteristics of viable splenic leucocytes. Flow cytometry was performed using a BD™ LSR II Flow Cytometer System.

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Preparation and Storage

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

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with BD HorizonTM BV421 under optimum conditions, and unconjugated antibody and free BD HorizonTM

BV421 were removed. **Application Notes**

Application

| Application | |
|------------------------------|------------------|
| Flow cytometry | Routinely Tested |
| Suggested Companion Products | |

| Catalog Number | Name | Size | Clone |
|----------------|--|--------|--------|
| 554656 | Stain Buffer (FBS) | 500 ml | (none) |
| 562602 | Brilliant Violet [™] 421 Rat IgG2a, κ Isotype Control | 50 µg | R35-95 |
| 555899 | Lysing Buffer | 100 ml | (none) |
| 553047 | FITC Rat Anti-Mouse CD4 | 0.5 mg | RM4-5 |
| 553046 | FITC Rat Anti-Mouse CD4 | 0.1 mg | RM4-5 |
| 561835 | FITC Rat Anti-Mouse CD4 | 25 μg | RM4-5 |
| 553031 | FITC Rat Anti-Mouse CD8a | 0.5 mg | 53-6.7 |
| 553030 | FITC Rat Anti-Mouse CD8a | 0.1 mg | 53-6.7 |
| 561966 | FITC Rat Anti-Mouse CD8a | 25 μg | 53-6.7 |

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.

- Source of all serum proteins is from USDA inspected abattoirs located in the United States. 2.
- 3. An isotype control should be used at the same concentration as the antibody of interest.
- Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols. 4.
- Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before 5. discarding to avoid accumulation of potentially explosive deposits in plumbing.
- For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at 6.
- Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR. 7
- Brilliant Violet[™] 421 is a trademark of Sirigen. 8.

www.bdbiosciences.com/colors.

References

Bourgeois C, Rocha B, Tanchot C. A role for CD40 expression on CD8+ T cells in the generation of CD8+ T cell memory. Science. 2002; 297(5589):2060-2063. (Biology)

Grewal IS, Flavell RA. CD40 and CD154 in cell-mediated immunity. Annu Rev Immunol. 1998; 16:111-135. (Biology)

Hasbold J, Johnson-Leger C, Atkins CJ, Clark EA, Klaus GG. Properties of mouse CD40: cellular distribution of CD40 and B cell activation by monoclonal anti-mouse CD40 antibodies. Eur J Immunol. 1994; 24(8):1835-1842. (Immunogen: Activation, (Co)-stimulatio)

Hasbold J, Klaus GG. B cells from CBA/N mice do not proliferate following ligation of CD40. Eur J Immunol. 1994; 24(1):152-157. (Clone-specific: Activation, (Co) -stimulatio)

Klaus GG, Holman M, Hasbold J. Properties of mouse CD40: the role of homotypic adhesion in the activation of B cells via CD40. Eur J Immunol. 1994; 24(11):2714-2719. (Biology)

Leifeld L, Trautwein C, Dumoulin FL, Manns MP, Sauerbruch T, Spengler U. Enhanced expression of CD80 (B7-1), CD86 (B7-2), and CD40 and their ligands CD28 and CD154 in fulminant hepatic failure. Am J Pathol. 1999; 154(6):1711-1720. (Biology: Immunohistochemistr)

Mahnke K. Becher P. Ricciardi-Castagnoli P. Luger TA. Schawrz T Grabbe S. CD14 is expressed by subsets of murine dendritic cells and upregulated by lipopolysaccharide. In: Ricciardi-Castagnoli P, ed. Dendritic Cells in Fundamental and Clinical Immunology. New York: Plenum Press; 1997:145-159. (Biology) Munder M, Mallo M, Eichmann K, Modolell M. Murine macrophages secrete interferon gamma upon combined stimulation with interleukin (IL)-12 and IL-18: A novel pathway of autocrine macrophage activation. J Exp Med. 1998; 187(12):2103-2108. (Biology)

Noelle RJ, Ledbetter JA, Aruffo A. CD40 and its ligand, an essential ligand-receptor pair for thymus-dependent B-cell activation. Immunol Today. 1992; 13(11):431-433. (Biology)

Parry SL, Hasbold J, Holman M, Klaus GG. Hypercross-linking surface IgM or IgD receptors on mature B cells induces apoptosis that is reversed by costimulation with IL-4 and anti-CD40. J Immunol. 1994; 152(6):2821-2829. (Biology)

Parry SL, Holman MJ, Hasbold J, Klaus GG. Plastic-immobilized anti-mu or anti-delta antibodies induce apoptosis in mature murine B lymphocytes. Eur J Immunol. 1994; 24(4):974-979. (Biology)

Tomura M, Yu WG, Ahn HJ, et al. A novel function of Valpha14+CD4+NKT cells: stimulation of IL-12 production by antigen-presenting cells in the innate immune system, J Immunol, 1999; 163(1):93-101, (Biology)

Turner JG, Rakhmilevich AL, Burdelya L, et al. Anti-CD40 antibody induces antitumor and antimetastatic effects: the role of NK cells. J Immunol. 2001; 166(1):89-94. (Biology)

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