Technical Data Sheet

BV650 Rat Anti-Mouse CD24

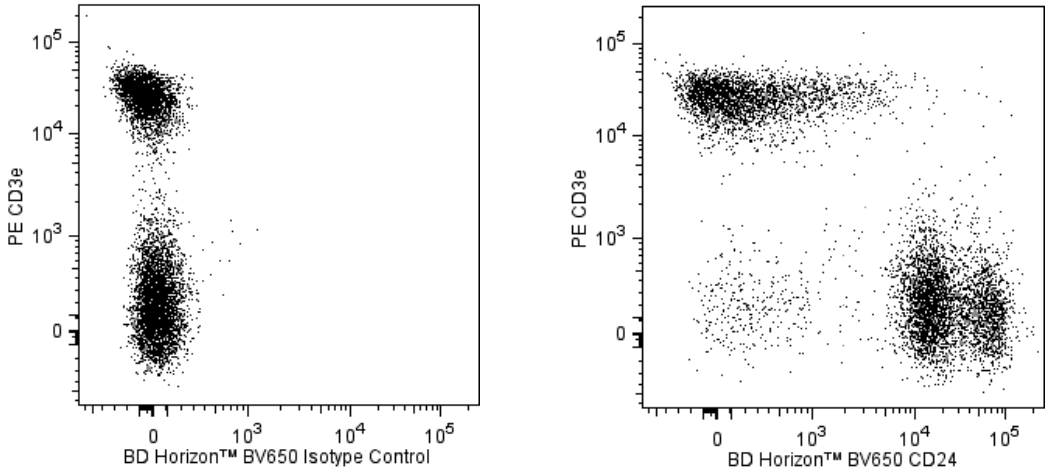
Product Information

Material Number:	**563545**
Alternate Name:	CD24a; HSA; Heat Stable Antigen; Ly-52; Nectadrin; R13-Ag
Size:	50 µg
Concentration:	0.2 mg/ml
Clone:	M1/69
Immunogen:	C57BL/10 Mouse Splenic T Lymphocytes
Isotype:	Rat (DA) IgG2b, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The M1/69 monoclonal antibody specifically binds to CD24 (Heat-Stable Antigen, HSA or HsAg), a variably glycosylated, glycosyl-phosphatidylinositol-anchored membrane protein expressed on erythrocytes, granulocytes, monocytes, lymphocytes, and neurons. Hematopoietic stem cells of the embryonic yolk sac and fetal liver express CD24. Levels of expression of CD24 vary during differentiation of the T and B cell lineages. In the bone marrow, hematopoietic progenitors acquire CD24 expression upon commitment to the B-lymphocyte lineage. Immature B cells in the bone marrow express low CD24 levels whereas peripheral B lymphocytes express intermediate to high levels of CD24. The level of CD24 expression has been reported to rise upon activation of splenic B cells with LPS, but not with CD154 (CD40 Ligand). The majority of thymocytes express high levels of CD24, while most mature thymic and peripheral T lymphocytes do not express CD24. In contrast, TCR-bearing thymocytes which emigrate to the spleen are CD24+. Dendritic cells of the thymus, spleen, liver, and epidermal Langerhans cells have also been reported to express CD24. CD24 is not expressed by NK cells, as determined by staining with J11d mAb (Cat. No. 553146). CD24 is involved in the costimulation of CD4+ T cells by B cells, it is a "co-inducer" of in vitro thymocyte maturation, and it is a ligand of CD62P (P-selectin). While the monoclonal antibodies 30-F1, M1/69, and J11d all react with CD24, they show subtle differences in the level of staining of different lymphocyte populations. When possible, investigators should continue to use the same monoclonal anti-CD24 antibody as used in previous studies.

The antibody was conjugated to BD Horizon™ BV650 which is part of the BD Horizon™ Brilliant Violet™ family of dyes. This dye is a tandem fluorochrome of BD Horizon™ BV421 with an Ex Max of 405-nm and an acceptor dye with an Em Max at 650-nm. BD Horizon™ BV650 can be excited by the violet laser and detected in a filter used to detect APC-like dyes (eg, 660/20-nm filter). Due to the excitation and emission characteristics of the acceptor dye, there will be spillover into the APC and Alexa Fluor® 700 detectors. However, the spillover can be corrected through compensation as with any other dye combination.



Two-color flow cytometric analysis of CD24 expression on mouse splenocytes. *Mouse splenic leucocytes were preincubated with Purified Rat Anti-Mouse CD16/CD32 antibody (Mouse BD Fc Block™) (Cat. No. 553141/553142). The cells were then stained with PE Hamster Anti-Mouse CD3e antibody (Cat. No. 553064/553063/561824) and either BD Horizon™ BV650 Rat IgG2b, κ Isotype Control (Cat. No. 563233, Left Panel) or with the BD Horizon™ BV650 Rat Anti-Mouse CD24 antibody (Cat. No. 563545, Right Panel). Two-color flow cytometric dot plots showing the correlated expression patterns of CD3e versus CD24 (or Ig Isotype control staining) were derived from gated events with the forward and side light-scatter characteristics of viable leucocytes. Flow cytometric analysis was performed using a BD™ LSR II Flow Cytometer System.*

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	800.979.9408	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**

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 Horizon™ BV650 under optimum conditions, and unconjugated antibody and free BD Horizon™ BV650 were removed.

Application Notes

Application

Flow cytometry	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
563233	BV650 Rat IgG2b, κ Isotype Control	50 µg	R35-38
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
553142	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.5 mg	2.4G2
555899	Lysing Buffer	100 ml	(none)
553064	PE Hamster Anti-Mouse CD3e	0.2 mg	145-2C11
553063	PE Hamster Anti-Mouse CD3e	0.1 mg	145-2C11
561824	PE Hamster Anti-Mouse CD3e	25 µg	145-2C11

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. An isotype control should be used at the same concentration as the antibody of interest.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
5. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
6. 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.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
8. Brilliant Violet™ 650 is a trademark of Sirigen.

References

Allman DM, Ferguson SE, Cancro MP. Peripheral B cell maturation. I. Immature peripheral B cells in adults are heat-stable antigenhi and exhibit unique signaling characteristics. *J Immunol.* 1992; 149(8):2533-2540. (Clone-specific: Flow cytometry, Fluorescence activated cell sorting)

Allman DM, Ferguson SE, Lentz VM, Cancro MP. Peripheral B cell maturation. II. Heat-stable antigen(hi) splenic B cells are an immature developmental intermediate in the production of long-lived marrow-derived B cells. *J Immunol.* 1993; 151(9):4431-4444. (Clone-specific: Flow cytometry, Fluorescence activated cell sorting)

Alterman LA, Crispe IN, Kinnon C. Characterization of the murine heat-stable antigen: an hematolymphoid differentiation antigen defined by the J11d, M1/69 and B2A2 antibodies. *Eur J Immunol.* 1990; 20(7):1597-1602. (Clone-specific: Flow cytometry)

Cibotti R, Punt JA, Dash KS, Sharrow SO, Singer A. Surface molecules that drive T cell development in vitro in the absence of thymic epithelium and in the absence of lineage-specific signals. *Immunity.* 1997; 6(3):245-255. (Clone-specific: (Co)-stimulation, Flow cytometry)

Crispe IN, Bevan MJ. Expression and functional significance of the J11d marker on mouse thymocytes. *J Immunol.* 1987 April; 138(7):2013-2018. (Clone-specific: Flow cytometry)

Milstein C, Galfre G, Secher DS, Springer T. Monoclonal antibodies and cell surface antigens. *Ciba Found Symp.* 1979; 66:251-276. (Clone-specific: Flow cytometry)

Springer T, Galfre G, Secher D, Milstein C. Monoclonal xenogeneic antibodies to mouse leukocyte antigens: identification of macrophage-specific and other differentiation antigens. *Curr Top Microbiol Immunol.* 1978; 81:45-50. (Immunogen: Radioimmunoassay)

Springer T, Galfre G, Secher DS, Milstein C. Monoclonal xenogeneic antibodies to murine cell surface antigens: identification of novel leukocyte differentiation antigens. *Eur J Immunol.* 1978; 8(8):539-551. (Immunogen: Blocking, Radioimmunoassay)

Stall AM, Wells SM. FACS analysis of murine B-cell populations. In: Herzenberg LA, Weir DM, Blackwell C, ed. *Weir's Handbook of Experimental Immunology.* Blackwell Science Publishers; 1997:63.1-63.17. (Clone-specific: Flow cytometry)

Veillette A, Zuniga-Pflucker JC, Bolen JB, Kruisbeek AM. Engagement of CD4 and CD8 expressed on immature thymocytes induces activation of intracellular tyrosine phosphorylation pathways. *J Exp Med.* 1989; 170(5):1671-1680. (Clone-specific: Cell separation, Cytotoxicity)

Wenger RH, Rochelle JM, Seldin MF, Kohler G, Nielsen PJ. The heat stable antigen (mouse CD24) gene is differentially regulated but has a housekeeping promoter. *J Biol Chem.* 1993; 268(31):23345-23352. (Biology)

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	800.979.9408	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**