

Technical Data Sheet

BV421 Rat Anti-Mouse Siglec-F

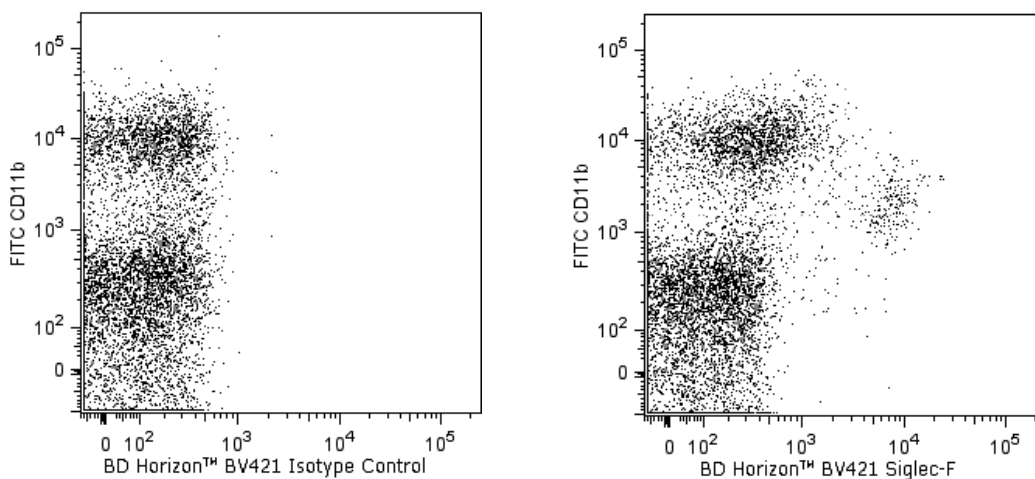
Product Information

Material Number:	562681
Alternate Name:	SiglecF; Siglec5; sialic acid binding Ig-like lectin 5; SIGL5; Siglec-5
Size:	50 µg
Concentration:	0.2 mg/ml
Clone:	E50-2440
Immunogen:	Mouse Siglec-F and human IgG Fc recombinant fusion protein
Isotype:	Rat (LOU) IgG2a, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The E50-2440 monoclonal antibody specifically recognizes Siglec-F. Siglecs are the sialic acid-binding immunoglobulin superfamily *lectins* defined in the human, each of which has a distinctive expression pattern in the hematopoietic system and at least some of which are known to mediate cell-cell interactions. Orthologous proteins of human Siglec-1 (Sialoadhesin or CD169), Siglec-2 (CD22), and Siglec-4 (myelin-associated glycoprotein) have been characterized in the mouse. Human Siglec-3 (CD33) and Siglecs-5 through -10 are encoded by a cluster of closely related genes, and each has two cytoplasmic ITIM (*I*mmunoreceptor *T*yrosine-based *I*nhibitory *M*otifs). Similarly, mouse Siglec-F is encoded by the *SiglecF* gene in a syntenic cluster in the mouse, and the protein has sialic acid-binding activity and an intracytoplasmic ITIM. Its expression pattern differs from those of the human Siglec-3-related proteins in that it is found on immature cells of the myelomonocytic lineage, with reduced expression on mature neutrophils and monocytes, and not on lymphoid cells. It has been proposed that mAb E50-2440 may be used for identification of immature myelomonocytic cells in the mouse.

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



Multicolor flow cytometric analysis of Siglec-F expression on BALB/c mouse bone marrow cells. Bone marrow cells were stained simultaneously with FITC Rat Anti-Mouse CD11b antibody (Cat. No. 553310/557396/561688) and with either BD Horizon™ BV421 Rat IgG2a, κ Isotype Control (Cat. No. 562602; Left Panel) or BD Horizon™ BV421 Rat Anti-Mouse Siglec-F (Cat. No. 562681/565934; Right Panel) in the presence of Purified Rat Anti-Mouse CD16/CD32 antibody (Mouse BD Fc Block™) (Cat. No. 553141/553142). Two-color flow cytometric dot plots show the correlated expression patterns of Siglec-F (or Ig isotype control staining) versus CD11b for gated events with the forward and side light-scatter characteristics of viable bone marrow cells. Flow cytometry was performed using a BD™ LSR II Flow Cytometer System.

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™ BV421 under optimum conditions, and unconjugated antibody and free BD Horizon BV421 were removed.

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Application Notes

Application

Flow cytometry

Routinely Tested

Recommended Assay Procedure:

For optimal and reproducible results, BD Horizon Brilliant Stain Buffer should be used anytime two or more BD Horizon Brilliant dyes (including BD Optibuild Brilliant reagents) are used in the same experiment. Fluorescent dye interactions may cause staining artifacts which may affect data interpretation. The BD Horizon Brilliant Stain Buffer was designed to minimize these interactions. More information can be found in the Technical Data Sheet of the BD Horizon Brilliant Stain Buffer (Cat. No. 563794/566349) or the BD Horizon Brilliant Stain Buffer Plus (Cat. No. 566385).

Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
562602	BV421 Rat IgG2a, κ Isotype Control	50 μ g	R35-95
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
553310	FITC Rat Anti-CD11b	0.5 mg	M1/70
557396	FITC Rat Anti-CD11b	0.1 mg	M1/70
561688	FITC Rat Anti-CD11b	25 μ g	M1/70
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
565934	BV421 Rat Anti-Mouse Siglec-F	25 μ g	E50-2440
563794	Brilliant Stain Buffer	100 Tests	(none)
566349	Brilliant Stain Buffer	1000 Tests	(none)
566385	Brilliant Stain Buffer Plus	1000 Tests	(none)

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. 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.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
6. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
7. BD Horizon Brilliant Stain Buffer is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,575,303; 8,354,239.
8. BD Horizon Brilliant Violet 421 is covered by one or more of the following US patents: 8,158,444; 8,362,193; 8,575,303; 8,354,239.
9. Please refer to www.bdbiosciences.com/pharming/protocols for technical protocols.

References

Angata T, Hingorani R, Varki NM, Varki A. Cloning and characterization of a novel mouse Siglec, mSiglec-F: differential evolution of the mouse and human (CD33) Siglec-3-related gene clusters. *J Biol Chem*. 2001; 276(48):45128-45136. (Immunogen: Flow cytometry, Fluorescence activated cell sorting)

Crocker PR, Varki A. Siglecs, sialic acids and innate immunity. *Trends Immunol*. 2001; 22(6):337-342. (Biology)