# **Technical Data Sheet**

# PE-CF594 Mouse Anti-Mouse Ly-49H

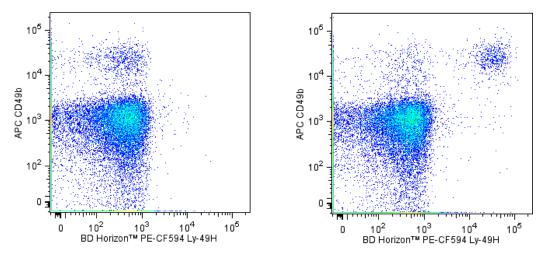
### **Product Information**

Material Number:	562519
Alternate Name:	Ly49h; Lymphocyte antigen 49H; Klra8; Cmv1; Cmv-1
Size:	50 µg
Concentration:	0.2 mg/ml
Clone:	3D10
Immunogen:	Mouse Ly-49A/H Transfected Cell Line
Isotype:	Mouse (BALB/c) IgG1
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA and $\leq 0.09\%$ sodium azide.

### Description

The 3D10 monoclonal antibody specifically binds to mouse Lymphocyte antigen 49H (Ly-49H; also known as Klra8 or Killer cell lectin-like receptor 8). The 3D10 antibody does not crossreact with related molecules such as Ly-49A, C, D or G2. Ly-49H is a type II transmembrane protein and a member of the Ly-49 C-type lectin multigene family of receptors expressed by NK cells. Cell surface Ly-49H is expressed by a subset of NK cells but not by NKT cells. Ly-49H is expressed by C57BL/6 and NWNA but not by BALB/c or DBA/2 mouse NK cells. Cell surface Ly-49H presents as a ~110 kDa disfulfide-linked homodimer and associates with signaling subunits such as DAP10 and DAP12 for optimal transduction of intracellular activation signals. Crosslinking of Ly-49H with the 3D10 antibody reportedly induces NK cell cytotoxicity and cytokine production. Ly-49H recognizes the mouse cytomegalovirus m157 glycoprotein that is expressed by infected cells and is required for protection against cytomegalovirus infection.

This antibody is conjugated to BD Horizon<sup>TM</sup> PE-CF594, which has been developed exclusively by BD Biosciences as a better alternative to PE-Texas Red®. PE-CF594 excites and emits at similar wavelengths to PE-Texas Red® yet exhibits improved brightness and spectral characteristics. Due to PE having maximal absorption peaks at 496 nm and 564 nm, PE-CF594 can be excited by the blue (488-nm), green (532-nm) and yellow-green (561-nm) lasers and can be detected with the same filter set as PE-Texas Red® (eg 610/20-nm filter).



Multicolor flow cytometric analysis of Ly-49H expression on BALB/c and C57BL/6 splenocytes. Mouse spleen cells from BALB/c (Left Panel) or C57BL/6 (Right Panel) mice were stained with BD Horizon™ PE-CF594 Mouse Anti-Mouse Ly-49H (Cat. No. 562519) and APC Rat Anti-Mouse CD49b (Clone DX5, Cat. No. 560628) antibodies. Two-color flow cytometric dot plots showing the correlated expression patterns of CD49b versus Ly-49H were derived from gated events with the forward and side light-scatter characteristics of viable lymphocytes. As expected, only C57BL/6 mouse splenocytes contained a subset of NK cells (Right Panel) that expressed Ly-49H whereas BALB/c splenic lymphocytes were Ly-49H-negative (Left Panel).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 Horizon™ PE-CF594 under optimum conditions, and unconjugated antibody and free PE-CF594

# were removed.

## **Application Notes**

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Flow cytometry	Routinel	y Tested		
Suggested Compa	nion Products			
Catalog Number	Name	Size	Clone	
554656	Stain Buffer (FBS)	500 ml	(none)	
562292	PE-CF594 Mouse IgG1, κ Isotype Control	0.1 mg	X40	
560628	APC Rat Anti-Mouse CD49b	50 µg	DX5	

### **Product Notices**

- Since applications vary, each investigator should titrate the reagent to obtain optimal results. 1.
- An isotype control should be used at the same concentration as the antibody of interest. 2.
- Source of all serum proteins is from USDA inspected abattoirs located in the United States. 3.
- 4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
- Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem 5. fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
- 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. Texas Red is a registered trademark of Molecular Probes, Inc., Eugene, OR.
- CFTM is a trademark of Biotium, Inc. 9.
- 10. When excited by the yellow-green (561-nm) laser, the fluorescence may be brighter than when excited by the blue (488-nm) laser.
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- 12. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using multi-laser cytometers, which may directly excite both PE and CF™594.

#### References

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Brown MG, Dokun AO, Heusel JW, et al. Vital involvement of a natural cell activation receptor in resistance to viral infection. Science. 2001; 292(5518):934-937. (Clone-specific: Activation, Bioassay, Blocking, Cytotoxicity, Flow cytometry)

Orr MT, Sun JC, Hesslein DG, et al. Ly49H signaling through DAP10 is essential for optimal natural killer cell responses to mouse cytomegalovirus infection. J Exp Med. 2009; 206(4):807-817. (Clone-specific: Blocking)

Silver ET, Elliott JF, Kane KP. Alternatively spliced Ly-49D and H transcripts are found in IL-2-activated NK cells. Immunogenetics. 1996; 44(6):478-482. (Biology) Smith HR, Chuang HH, Wang LL, Salcedo M, Heusel JW, Yokoyama WM. Nonstochastic Coexpression of activation receptors on murine Natural Killer cells. J Exp Med. 2000; 191(8):1341-1354. (Immunogen: Activation, Cytotoxicity, Flow cytometry, Immunoprecipitation)

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