## **Technical Data Sheet**

# **BV605 Hamster Anti-Mouse CD27**

## **Product Information**

Material Number:	563365
Alternate Name:	Tnfrsf7; Tumor necrosis factor receptor superfamily member 7; Tp55; S152
Size:	50 µg
Concentration:	0.2 mg/ml
Clone:	LG.3A10
Immunogen:	Armenian hamster fibroblast line ARHO12 transfected with mouse Cd27 cDNA
Isotype:	Armenian Hamster IgG1, κ
Reactivity:	QC Testing: Mouse
	Tested in Development: Rat
Storage Buffer:	Aqueous buffered solution containing BSA and $\leq 0.09\%$ sodium azide.

## Description

The LG.3A10 monoclonal antibody specifically binds to CD27, a lymphocyte-restricted member of the Tumor Necrosis Factor Receptor family which binds to CD70. The CD27 molecule is a 45-kDa transmembrane glycoprotein which is constitutively expressed by lymphocytes of the T lineage: virtually all thymocytes and over 90% of peripheral T cells bearing both  $\alpha\beta$  and  $\gamma\delta$  T-cell receptors. CD27 cooperates with the pre-TCR in mediating thymocyte differentiation and expansion. In addition, one to ten percent of mature peripheral B cells express CD27, and CD27's role in the differentiation of human plasma cells has been studied. Mouse NK cells, freshly isolated and IL-2-activated, also express CD27. In the bone marrow, CD27 is found on a progenitor population which provides short-term hematopoietic reconstitution. Cells of the myeloid lineage do not express CD27. Cross-linked LG.3A10 mAb has been reported to amplify the proliferative response of purified T lymphocytes to suboptimal mitogenic stimulation1 and to enhance NK-cell proliferation and IFN- $\gamma$  production. In contrast, non-cross-linked LG.3A10 mAb inhibits CD3-induced pre-T cell development by interfering with the receptor-ligand interaction. This hamster mAb to a mouse leukocyte antigen has been observed to cross-react with a similar population of rat leukocytes.

This antibody is conjugated to BD Horizon BV605 which is part of the BD Horizon Brilliant<sup>™</sup> Violet family of dyes. With an Ex Max of 407-nm and Em Max of 602-nm, BD Horizon BV605 can be excited by a violet laser and detected with a standard 610/20-nm filter set. BD Horizon BV605 is a tandem fluorochrome of BD Horizon BV421 and an acceptor dye with an Em max at 605-nm. Due to the excitation of the acceptor dye by the green (532 nm) and yellow-green (561 nm) lasers, there will be significant spillover into the PE and BD Horizon PE-CF594 detectors off the green or yellow-green lasers. BD Horizon BV605 conjugates are very bright, often exhibiting brightness equivalent to PE conjugates and can be used as a third color off of the violet laser.

For optimal and reproducible results, BD Horizon Brilliant Stain Buffer should be used anytime two or more BD Horizon Brilliant dyes 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).

## **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<sup>™</sup> BV605 under optimum conditions, and unconjugated antibody and free BD Horizon<sup>™</sup> BV605 were removed.

## **Application Notes**

#### Application

Flow cytometry	Routinely Tested				
Suggested Compar	nion Products				
Catalog Number	Name	Size	Clone		
54656	Stain Buffer (FBS)	500 mL	(none)		
63009	BV605 Hamster IgG1, κ Isotype Control	50 µg	A19-3		
55899	Lysing Buffer	100 mL	(none)		
53141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2		
53142	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.5 mg	2.4G2		
53066	APC Hamster Anti-Mouse CD3e	0.1 mg	145-2C11		
61826	APC Hamster Anti-Mouse CD3e	25 μg	145-2C11		
63794	Brilliant Stain Buffer	5 mL	(none)		

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Flow cytometric analysis of CD27 expression on mouse splenocytes. BALB/c 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 APC Rat Anti-Mouse CD3e antibody (Cat. No. 553066/561826) and either BD Horizon™ BV605 Hamster IgG1, κ Isotype Control (Cat. No. 563009; Left Panel) or BD Horizon™ BV605 Hamster Anti-Mouse CD27 antibody (Cat. No. 563365; Right Panel). Two-color flow cytometric dot plots showing the correlated expression patterns of CD27 (or Ig Isotype control staining) versus CD3e 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.

## **Product Notices**

- Since applications vary, each investigator should titrate the reagent to obtain optimal results. 1.
- 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. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem 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.
- Caution: Sodium azide vields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before 6. 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 7. www.bdbiosciences.com/colors.
- 8. Although every effort is made to minimize the lot-to-lot variation in the efficiency of the fluorochrome energy transfer, differences in the residual emission from BD Horizon™ BV421 may be observed. Therefore, we recommend that individual compensation controls be performed for every BD Horizon<sup>™</sup> BV605 conjugate.
- Species testing during development may have been performed with a different format of the same clone. Selected applications have been 9. tested for cross-reactivity.
- 10. Although hamster immunoglobulin isotypes have not been well defined, BD Biosciences Pharmingen has grouped Armenian and Syrian hamster IgG monoclonal antibodies according to their reactivity with a panel of mouse anti-hamster IgG mAbs. A table of the hamster IgG groups, Reactivity of Mouse Anti-Hamster Ig mAbs, may be viewed at http://www.bdbiosciences.com/documents/hamster chart 11x17.pdf.
- 11. CF<sup>TM</sup> is a trademark of Biotium, Inc.

#### References

Agematsu K, Hokibara S, Nagumo H, Shinozaki K, Yamada S, Komiyama A. Plasma cell generation from B-lymphocytes via CD27/CD70 interaction. Leuk Lymphoma. 1999; 35(3-4):219-225. (Biology)

Gravestein LA, Blom B, Nolten LA, et al. Cloning and expression of murine CD27: comparison with 4-1BB, another lymphocyte-specific member of the nerve growth factor receptor family. Eur J Immunol. 1993; 23(4):943-950. (Biology)

Gravestein LA, Nieland JD, Kruisbeek AM, Borst J. Novel mAbs reveal potent co-stimulatory activity of murine CD27. Int Immunol. 1995; 7(4):551-557.

(Immunogen: (Co)-stimulation, Flow cytometry, Functional assay, Immunofluorescence, Immunoprecipitation)

Gravestein LA, van Ewijk W, Ossendorp F, Borst J. CD27 cooperates with the pre-T cell receptor in the regulation of murine T cell development. J Exp Med. 1996; 184(2):675-685. (Clone-specific: Flow cytometry, Immunofluorescence, Inhibition)

Takeda K, Oshima H, Hayakawa Y, et al. CD27-mediated activation of murine NK cells. J Immunol. 2000; 164(4):1741-1745. (Clone-specific: (Co)-stimulation, Enhancement, Flow cytometry)

Wiesmann A, Phillips RL, Mojica M, et al. Expression of CD27 on murine hematopoietic stem and progenitor cells. Immunity. 2000; 12(2):193-199. (Clone-specific: Flow cytometry)

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