Technical Data Sheet

BB515 Mouse Anti-Human CD335 (NKp46)

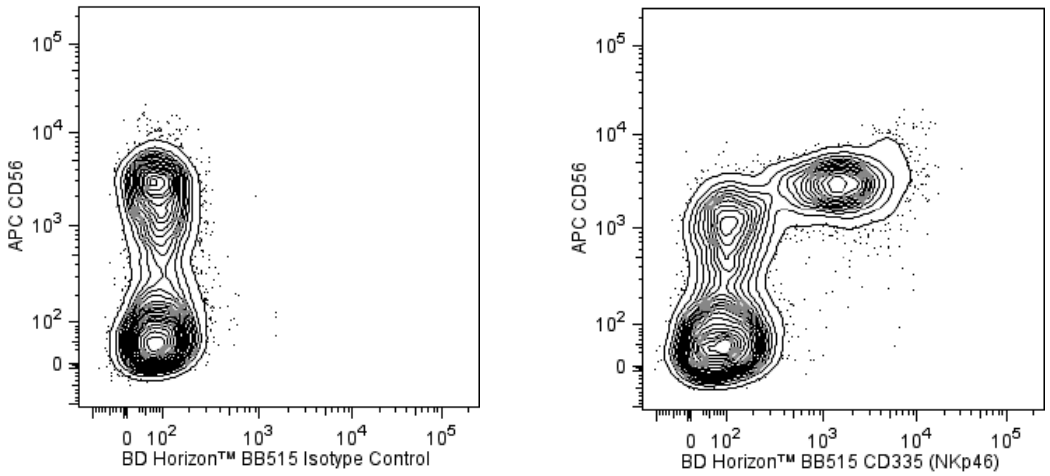
Product Information

Material Number:	**564536**
Alternate Name:	NCR1; NK-p46; hNKp46; LY94; Natural cytotoxicity triggering receptor 1
Size:	100 Tests
Vol. per Test:	5 µl
Clone:	9E2/NKp46 (also known as 9-E2)
Immunogen:	Human NKp46 Recombinant Protein
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Human
Workshop:	VIII 80442
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The 9E2/Nkp46 monoclonal antibody specifically binds to CD335. CD335 is also known as the Natural killer cell p46-related protein (NKp46) and the Natural cytotoxicity triggering receptor 1 (NCR1). CD335 is a 46 kDa type I membrane glycoprotein that is expressed on resting and activated NK cells. Its extracellular region contains two C2-type, Ig-like domains. The transmembrane domain contains a positively charged amino acid (Arg) which could be involved in stabilizing the association with CD3ζ. Its intracellular region does not contain immunoreceptor tyrosine-based activating motifs (ITAM), but it is linked to intracytoplasmic transduction machinery by its association with CD3ζ and FcεRIγ adaptor proteins. CD335 along with NKp30 and NKp44 are referred to as natural cytotoxicity receptors (NCR). These receptors play very important roles in cells that kill virus-infected target cells, tumor cells and MHC-class I-unprotected cells.

The antibody was conjugated to BD Horizon BB515 which is part of the BD Horizon Brilliant™ Blue family of dyes. With an Ex Max near 490 nm and an Em Max near 515 nm, BD Horizon BB515 can be excited by the blue laser (488 nm) laser and detected with a 530/30 nm filter. This dye has been exclusively developed by BD Biosciences and is up to seven times brighter than FITC with less spillover into the PE channel. Due to similar excitation and emission properties, BB515, FITC, and Alexa Fluor® 488 cannot be used simultaneously. It is not recommended to use BB515 in cocktails that include Streptavidin conjugates as it may cause high background.



Two-color flow cytometric analysis of CD335 (NKp46) expression on human peripheral blood lymphocytes. *Whole blood was stained with APC Mouse Anti-Human CD56 antibody (Cat. No. 555518) and either BD Horizon™ BB515 Mouse IgG1, κ Isotype Control (Cat. No. 564416; Left Panel) or BD Horizon BB515 Mouse Anti-Human CD335 (NKp46) antibody (Cat. No . 564536/564537; Right Panel). Erythrocytes were lysed with BD FACSLyse™ Lysing Buffer (Cat. No. 349202). Two-color flow cytometric contour plots showing the correlated expression of CD335 (NKp46) [or Ig Isotype control staining] versus CD56 were derived from gated events with the forward and side light -scatter characteristics of intact lymphocytes. Flow cytometric analysis 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™ BB515 under optimum conditions and unconjugated antibody was removed.

Application Notes

Application

Flow cytometry	Routinely Tested

Recommended Assay Procedure:

BD™ CompBeads can be used as surrogates to assess fluorescence spillover (Compensation). When fluorochrome conjugated antibodies are bound to CompBeads, they have spectral properties very similar to cells. However, for some fluorochromes there can be small differences in spectral emissions compared to cells, resulting in spillover values that differ when compared to biological controls. It is strongly recommended that when using a reagent for the first time, users compare the spillover on cells and CompBead to ensure that BD Comp beads are appropriate for your specific cellular application.

For optimal results, it is recommended to perform 2 washes after staining with antibodies. Cells may be prepared, stained with antibodies and washed twice with wash buffer per established protocols for immunofluorescence staining, prior to acquisition on a flow cytometer. Performing fewer than the recommended wash steps may lead to increased spread of the negative population.

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. 563794/566349) or the BD Horizon Brilliant Stain Buffer Plus (Cat. No. 566385).

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
563794	Brilliant Stain Buffer	100 Tests	(none)
564416	BB515 Mouse IgG1, κ Isotype Control	100 µg	X40
564537	BB515 Mouse Anti-Human CD335 (NKp46)	25 Tests	9E2/NKp46
555518	APC Mouse Anti-Human CD56	100 Tests	B159
349202	BD FACS™ Lysing Solution	100 mL	(none)
555899	Lysing Buffer	100 mL	(none)
566349	Brilliant Stain Buffer	1000 Tests	(none)
566385	Brilliant Stain Buffer Plus	1000 Tests	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. An isotype control should be used at the same concentration as the antibody of interest.
3. 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.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
5. 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.
6. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Mandelboim O, Porgador A. NKp46. *Int J Biochem Cell Biol.* 2001; 33(12):1147-1150. (Biology)

Nakajima H, Cella M, Bouchon A, et al. Patients with X-linked lymphoproliferative disease have a defect in 2B4 receptor-mediated NK cell cytotoxicity. *Eur J Immunol.* 2000; 30(11):3309-3318. (Immunogen: Flow cytometry, Functional assay)

Sivori S, Pende D, Bottino C, et al. NKp46 is the major triggering receptor involved in the natural cytotoxicity of fresh or cultured human NK cells. Correlation between surface density of NKp46 and natural cytotoxicity against autologous, allogeneic or xenogeneic target cells. *Eur J Immunol.* 1999; 29(5):1656-1666. (Biology)

Sivori S, Vitale M, Morelli L, et al. p46, a novel natural killer cell-specific surface molecule that mediates cell activation. *J Exp Med.* 1997; 186(7):1129-1136. (Biology)