

## Technical Data Sheet

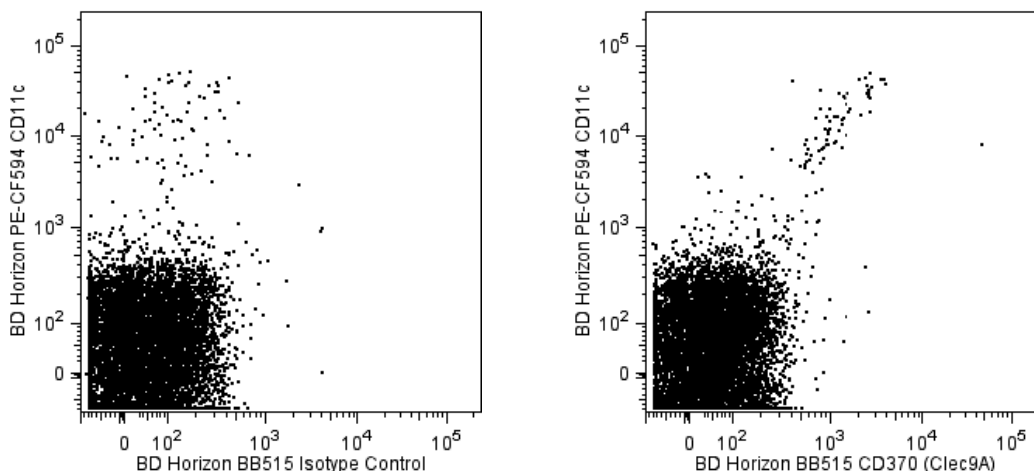
**BB515 Rat Anti-Mouse CD370 (Clec9A)****Product Information**

<b>Material Number:</b>	<b>565320</b>
<b>Alternate Name:</b>	CD370; CLC9A; Clec9a; DNGR-1; C-type lectin domain family 9 member A
<b>Size:</b>	50 µg
<b>Concentration:</b>	0.2 mg/ml
<b>Clone:</b>	10B4 (also known as 24/04-10B4)
<b>Immunogen:</b>	Mouse Clec9A Peptide
<b>Isotype:</b>	Rat (WI) IgG2a, κ
<b>Reactivity:</b>	QC Testing: Mouse
<b>Storage Buffer:</b>	Aqueous buffered solution containing ≤0.09% sodium azide.

**Description**

The 10B4 monoclonal antibody specifically binds to mouse Clec9A. Mouse Clec9A (C-type lectin domain family member 9A) is also known as DNGR1 (Dendritic cell natural killer lectin group receptor 1). It is a type II membrane protein with a single extracellular C-type lectin domain. Clec9A is a dendritic cell subtype-restricted C-type lectin-like receptor. Clec9A is selectively expressed on plasmacytoid dendritic cells and CD8<sup>+</sup> myeloid dendritic cells. Clec9A reportedly serves as a receptor for necrotic cells. It can mediate the cross-presentation of dead-cell associated antigens in a Syk-dependent manner.

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.



**Multicolor flow cytometric analysis of CD370 (Clec9A) 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 APC Rat Anti-Mouse CD4 (Cat. No. 553051/561091), BD Horizon™ BUV737 Rat Anti-Mouse CD8a (Cat. No. 564297), BD Horizon PE-CF594 Hamster Anti-Mouse CD11c (Cat. No. 562454) antibodies and either BD Horizon BB515 Rat IgG2a κ Isotype Control (Cat. No. 564418; Left Panel) or BD Horizon BB515 Rat Anti-Mouse CD370 (Clec9A) antibody (Cat. No. 565320/566033; Right Panel). Two color flow cytometric dot plots showing the correlated expression of CD11c and CD370 (Clec9A) [or Ig Isotype control staining] were derived from CD4- CD8a+ gated events with the forward and side light-scatter characteristics of viable splenic leucocytes. Flow cytometric analysis 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™ BB515 under optimum conditions and unconjugated antibody was removed.

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565320 Rev. 3



## 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 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).

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.

### 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)
564418	BB515 Rat IgG2a, κ Isotype Control	0.1 mg	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
564297	BUV737 Rat Anti-Mouse CD8a	50 µg	53-6.7
562454	PE-CF594 Hamster Anti-Mouse CD11c	0.1 mg	HL3
553051	APC Rat Anti-Mouse CD4	0.1 mg	RM4-5
561091	APC Rat Anti-Mouse CD4	25 µg	RM4-5
566349	Brilliant Stain Buffer	1000 Tests	(none)
566385	Brilliant Stain Buffer Plus	1000 Tests	(none)
566033	BB515 Rat Anti-Mouse CD370 (Clec9A)	25 µg	10B4

### Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
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](http://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 <http://regdocs.bd.com> to access safety data sheets (SDS).
7. Please refer to [www.bdbiosciences.com/us/s/resources](http://www.bdbiosciences.com/us/s/resources) for technical protocols.

### References

Caminschi L, Proietto AL, Ahmet F, et al. The dendritic cell subtype restricted C-type lectin Clec9A is a target for vaccine enhancement. *Blood*. 2008; 112(8):3264-3273. (Immunogen: Flow cytometry, Functional assay, Immunohistochemistry, In vivo exacerbation)

Huysamen C, Willment JA, Dennehy KM, Brown GD. CLEC9A is a novel activation C-type lectin-like receptor expressed on BDCA3+ dendritic cells and a subset of monocytes. *J Biol Chem*. 2008; 283(24):16693-16701. (Biology)

Sancho D, Joffre OP, Keller AM, et al. Identification of a dendritic cell receptor that couples sensing of necrosis to immunity. *Nature*. 2009; 458(7240):899-903. (Biology)