

## Technical Data Sheet

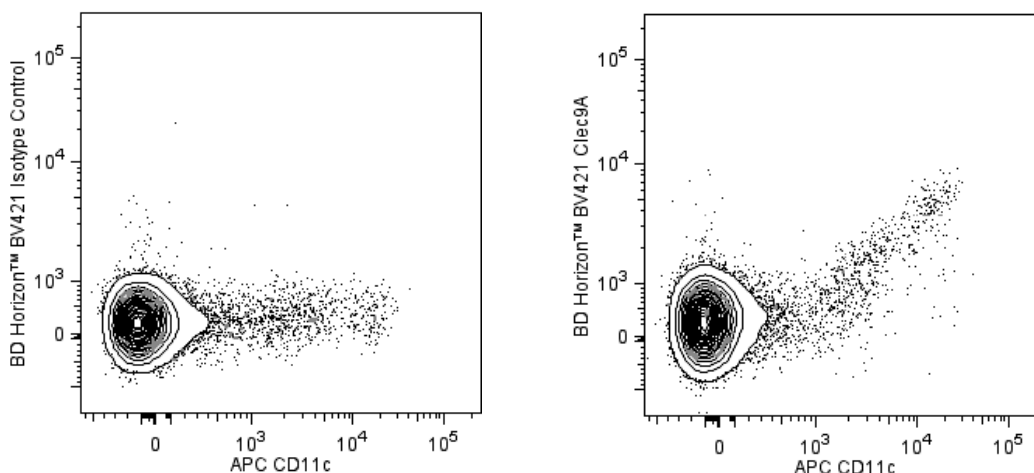
**BV421 Rat Anti-Mouse CD370 (Clec9A)****Product Information**

<b>Material Number:</b>	<b>564271</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 BSA and ≤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 BV421 which is part of the BD Horizon Brilliant™ Violet family of dyes. With an Ex Max near 407 nm and Em Max near 421 nm, BD Horizon BV421 can be excited by the violet laser (405 nm) and detected with a 450/50 nm filter. BD Horizon BV421 conjugates are very bright, often exhibiting a 10 fold improvement in brightness compared to Pacific Blue™ conjugates. Due to nearly identical excitation and emission properties but different spillover characteristics, BD Horizon BV421, Pacific Blue, and BD Horizon V450 cannot be used simultaneously.



**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 PE Rat Anti-Mouse CD4 (Cat. No. 553048/553049/561837), FITC Rat Anti-Mouse CD8a (Cat. No. 553031/553030/561966), APC Hamster Anti-Mouse CD11c (Cat. No. 550261/561119) antibodies and either BD Horizon™ BV421 Rat IgG2a κ Isotype Control (Cat. No. 562602; Left Panel) or BD Horizon BV421 Rat Anti-Mouse CD370 (Clec9A) antibody (Cat. No. 564271; Right Panel). Two color flow cytometric contour plots showing the correlated expression of CD11c and Clec9A (or Ig Isotype control staining) were derived from CD4- CD8a<sup>+</sup> gated events with the forward and side light-scatter characteristics of viable cells. 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 to the dye under optimum conditions and unconjugated antibody and free dye were removed.

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## 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).

### Suggested Companion Products

Catalog Number	Name	Size	Clone
562602	BV421 Rat IgG2a, κ Isotype Control	50 µg	R35-95
555899	Lysing Buffer	100 mL	(none)
553048	PE Rat Anti-Mouse CD4	0.1 mg	RM4-5
553049	PE Rat Anti-Mouse CD4	0.2 mg	RM4-5
561837	PE Rat Anti-Mouse CD4	25 µg	RM4-5
553031	FITC Rat Anti-Mouse CD8a	0.5 mg	53-6.7
553030	FITC Rat Anti-Mouse CD8a	0.1 mg	53-6.7
561966	FITC Rat Anti-Mouse CD8a	25 µg	53-6.7
550261	APC Hamster Anti-Mouse CD11c	0.1 mg	HL3
561119	APC Hamster Anti-Mouse CD11c	25 µg	HL3
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
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
566349	Brilliant Stain Buffer	1000 Tests	(none)
566385	Brilliant Stain Buffer Plus	1000 Tests	(none)
565445	Brilliant Stain Buffer	2.5 mL	(none)

### 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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
6. 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).
7. 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.
8. 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.
9. Please refer to <http://regdocs.bd.com> to access safety data sheets (SDS).
10. 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)