Technical Data Sheet

# BV421 Rat Anti-Mouse I-A/I-E

## Product Information

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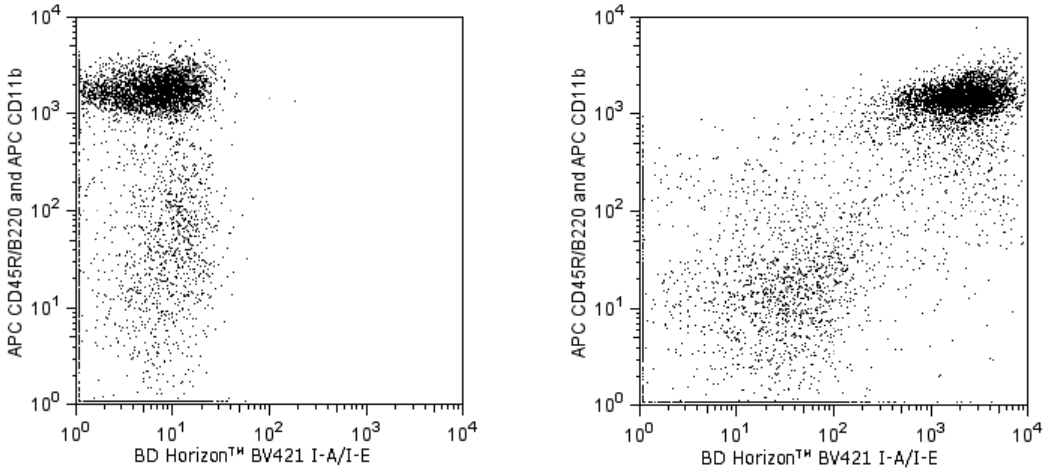
|---|---|
| **Material Number:** | **562564** |
| **Alternate Name:** | M5/114; H-2I; Ia Ag; I-Ab, I-Ad, I-Aq, I-Ed, and I-Ek MHC class II alloAgs |
| **Size:** | 50 µg |
| **Concentration:** | 0.2 mg/ml |
| **Clone:** | M5/114.15.2 (also known as M5/114) |
| **Immunogen:** | Activated C57BL/6 Mouse Spleen Cells |
| **Isotype:** | Rat (BN x LEW) IgG2b, κ |
| **Reactivity:** | QC Testing: Mouse |
| **Storage Buffer:** | Aqueous buffered solution containing BSA and ≤0.09% sodium azide. |

## Description

The M5/114.15.2 monoclonal antibody recognizes a polymorphic determinant shared by the I-A[b], I-A[d], I-A[q], I-E[d], and I-E[k] (but not I-A[f], I-A[k], or I-A[s]) MHC class II alloantigens that can be expressed by B cells, dendritic cells, monocytes, macrophages and activated T cells. It also reacts with cells from mice of the H-2[p] and H-2[r] haplotypes, and it is non-reactive with cells from NOD (H-2[g7]) mice. Flow cytometric analysis indicates that the M5/114.15.2 and 2G9 monoclonal antibodies have comparable reactivity on cells from mice with I-A[b], I-A[d], I-A[g7], I-A[q], I-E[d], and I-E[k] alloantigens.

The antibody was conjugated to BD Horizon™ BV421 which is part of the BD Horizon™ Brilliant Violet™ family of dyes. With an Ex Max of 407-nm and Em Max at 421-nm, BD Horizon™ BV421 can be excited by the violet laser and detected in the standard Pacific Blue™ filter set (eg, 450/50-nm filter). BD Horizon™ BV421 conjugates are very bright, often exhibiting a 10 fold improvement in brightness compared to Pacific Blue™ conjugates.




***Multicolor flow cytometric analysis of I-A/I-E MHC class II alloantigen expression on splenocytes from positive and negative mouse strains.*** *Mouse spleen cells from either M5/114-negative SJL (Left Panel) or M5/114-positive BALB/c (Right Panel) mice were stained with BD Horizon™ BV421 Rat Anti-Mouse I-A/I-E (Cat. No. 562564), APC Anti-Mouse CD45R/B220 (Cat. No. 553092/561880) and APC Anti-Mouse CD11b (Cat. No. 553312/561690) antibodies. Two-color flow cytometric dot plots showing the expression of I-A/I-E MHC class II alloantigens versus CD45R/B220 and CD11b were derived from gated events with the forward and side light-scatter characteristics of viable splenocytes. The M5/114 monoclonal antibody detects I-Ad and I-Ed MHC class II alloantigens that are expressed on both B cells and macrophages. 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™ BV421 under optimum conditions, and unconjugated antibody and free BD Horizon™ BV421 were removed.

## Application Notes

**Application**

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| Flow cytometry | Routinely Tested |

## Suggested Companion Products

| Catalog Number | Name | Size | Clone |
|---|---|---|---|
| 554656 | Stain Buffer (FBS) | 500 ml | (none) |
| 562603 | BV421 Rat IgG2b, κ Isotype Control | 50 µg | R35-38 |
| 553092 | APC Rat Anti-Mouse CD45R/B220 | 0.1 mg | RA3-6B2 |
| 561880 | APC Rat Anti-Mouse CD45R/B220 | 25 µg | RA3-6B2 |
| 553312 | APC Rat Anti-Mouse CD11b | 0.1 mg | M1/70 |
| 561690 | APC Rat Anti-Mouse CD11b | 25 µg | M1/70 |

## Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
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. 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.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
8. Brilliant Violet™ 421 is a trademark of Sirigen.

## References


Bhattacharya A, Dorf ME, Springer TA. A shared alloantigenic determinant on Ia antigens encoded by the I-A and I-E subregions: evidence for I region gene duplication. *J Immunol.* 1981; 127(6):2488-2495. (Immunogen: Immunoprecipitation)

Ernst DN, McQuitty DN, Weigle WO, Hobbs MV. Expression of membrane activation antigens on murine B lymphocytes stimulated with lipopolysaccharide. *Cell Immunol.* 1988; 114(1):161-173. (Clone-specific: Flow cytometry)

Guo MW, Watanabe T, Mori E, Mori T. Molecular structure and function of CD4 on murine egg plasma membrane. *Zygote.* 1995; 3(1):65-73. (Clone-specific: Blocking)

Hattori M, Buse JB, Jackson RA, et al. The NOD mouse: recessive diabetogenic gene in the major histocompatibility complex. *Science.* 1986; 231(4739):733-735. (Clone-specific)

Nelson AJ, Hosier S, Brady W, Linsley PS, Farr AG. Medullary thymic epithelium expresses a ligand for CTLA4 in situ and in vitro. *J Immunol.* 1998; 151(5):2453-2461. (Clone-specific: Blocking, Immunofluorescence, Immunohistochemistry)

Viville S, Neefjes J, Lotteau V, et al. Mice lacking the MHC class II-associated invariant chain. *Cell.* 1993; 72(4):635-648. (Clone-specific: Flow cytometry, Immunofluorescence)

Yamashita I, Nagata T, Tada T, Nakayama T. CD69 cell surface expression identifies developing thymocytes which audition for T cell antigen receptor-mediated positive selection. *Int Immunol.* 1993; 5(9):1139-1150. (Clone-specific: Blocking)



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