Technical Data Sheet

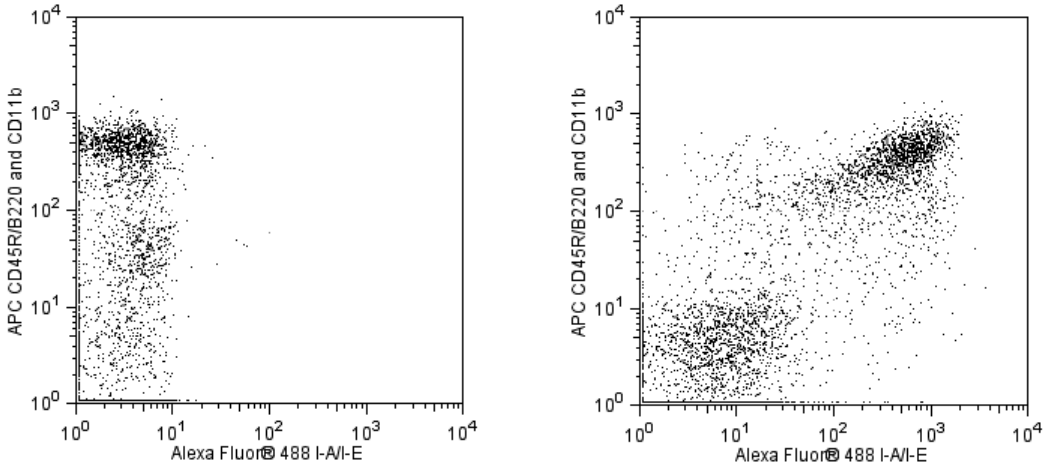
Alexa Fluor® 488 Rat Anti-Mouse I-A/I-E

Product Information

Material Number:	**562352**
Alternate Name:	I-Ab, I-Ad, I-Aq, I-Ed, and I-Ek MHC class II alloantigens
Size:	50 µg
Concentration:	0.2 mg/ml
Clone:	M5/114.15.2
Immunogen:	Activated C57BL/6 Mouse Spleen Cells
Isotype:	Rat (BN x LEW) IgG2b, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤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. 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 (Cat. No. 553621) 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.



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 Alexa Fluor® 488 Rat Anti-Mouse I-A/I-E (Cat. No. 562352), APC Rat Anti-Mouse CD45R/B220 (Cat. No. 553092/561880) and APC Rat 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.*

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 Alexa Fluor® 488 under optimum conditions, and unreacted Alexa Fluor® 488 was removed.

Application Notes

Application

Flow cytometry	Routinely Tested

BD Biosciences

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Suggested Companion Products

Catalog Number	Name	Size	Clone
557726	Alexa Fluor® 488 Rat IgG2b, κ Isotype Control	0.1 mg	A95-1
554656	Stain Buffer (FBS)	500 ml	(none)
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. An isotype control should be used at the same concentration as the antibody of interest.
3. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
4. Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
5. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
6. 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.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
8. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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)

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: Flow cytometry)

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: Immunofluorescence, Immunohistochemistry)

Shi Y, Kaliyaperumal A, Lu L, et al. Promiscuous presentation and recognition of nucleosomal autoepitopes in lupus: role of autoimmune T cell receptor alpha chain. *J Exp Med.* 1998; 187(3):367-378. (Clone-specific: Blocking)

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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