

## Technical Data Sheet

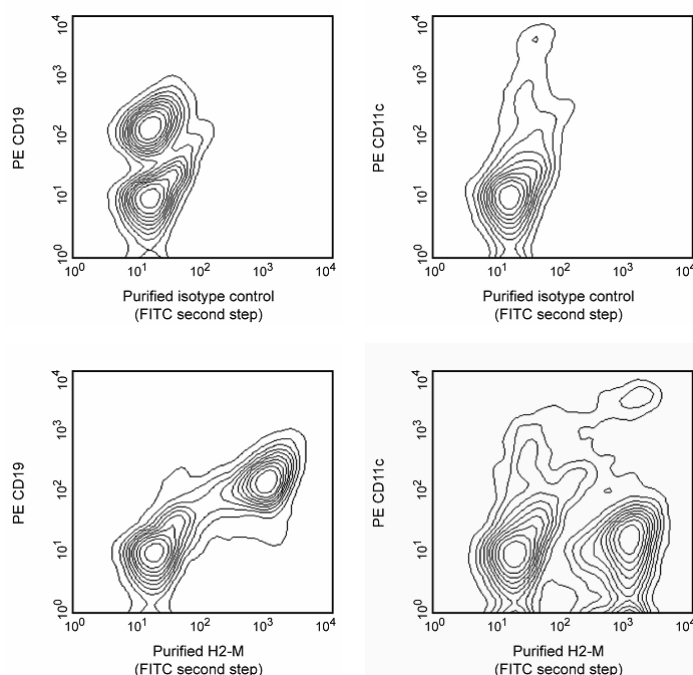
## Purified Rat Anti-Mouse H2-M

## Product Information

<b>Material Number:</b>	<b>552405</b>
<b>Alternate Name:</b>	H2-DM
<b>Size:</b>	0.1 mg
<b>Concentration:</b>	0.5 mg/ml
<b>Clone:</b>	2E5A
<b>Immunogen:</b>	Recombinant H2-DM protein
<b>Isotype:</b>	Rat IgG1, $\kappa$
<b>Reactivity:</b>	QC Testing: Mouse
<b>Storage Buffer:</b>	Aqueous buffered solution containing $\leq 0.09\%$ sodium azide.

## Description

H2-M, also known as H2-DM, is a non-classical MHC class II molecule in antigen-presenting cells. H2-M and CD74 (Ii) are critical components of the antigen-processing pathway of the classical MHC class II molecules. Like classical MHC class II molecules, non-classical MHC molecules have limited polymorphism. There are two isoforms of H2-M,  $\alpha\beta 1$  and  $\alpha\beta 2$ , encoded by the H2-DMA gene and either H2-DMb1 or H2-DMb2, respectively. The 2E5A antibody reacts with H2-M  $\alpha\beta 2$  dimers (the predominant form transcribed in the mouse spleen), but not  $\alpha\beta 1$  dimers. H2-M dimers (both isoforms) are integral proteins of lysosomal membranes, where they catalyze the release of CLIP (class II-associated Ii peptide) from the peptide-binding groove of classical MHC class II dimers and stabilize the open binding site to allow loading of exogenous peptides. They also may facilitate the selection of high-affinity antigenic peptides by allowing the dissociation of poorly fitting non-CLIP peptides to be exchanged for higher affinity peptides. This peptide-exchange function of H2-M is essential for the intrathymic development of the repertoire of CD4<sup>+</sup> T lymphocytes and the maturation of humoral and cell-mediated immune responses. H2-M is believed to be expressed in all cells which express classical MHC class II molecules, including peripheral B lymphocytes, macrophages, dendritic cells, thymic cortical epithelium, and some tumor cell lines. Its level of expression is at least partially controlled by CD74 (Ii), whereas its functional activity is negatively regulated by another non-classical MHC class II molecule, H2-O.



**Two-color analysis of the cytoplasmic expression of H2-M in splenic B lymphocytes and dendritic cells.** Fixed and permeabilized C57BL/6 splenocytes were stained with either purified rat IgG1,  $\kappa$  isotype control mAb R3-34 (Cat. No. 553922, top panels) or purified mAb 2E5A (bottom panels) in the presence of Mouse Fc Block™ (purified anti-mouse CD16/CD32 mAb 2.4 G2, Cat. No. 553141/553142), followed by FITC-conjugated anti-rat IgG1 mAb RG11/39.4 (Cat. No. 553892). B lymphocytes were identified by staining with PE-conjugated anti-mouse CD19 mAb 1D3 (Cat. No. 557399/553786, left panels), and dendritic cells were identified with PE-conjugated anti-mouse CD11c mAb HL3 (Cat. No. 557401/553802, right panels). Flow cytometry was performed on a BD FACSCalibur™ System (BD Biosciences, San Jose, CA).

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## Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Store undiluted at 4°C.

## Application Notes

### Application

Intracellular staining (flow cytometry)	Routinely Tested
Immunoprecipitation	Reported

### Recommended Assay Procedure:

For flow cytometry of leukocytes, it is recommended that Mouse Fc Block™ (purified anti-mouse CD16/CD32 mAb 2.4G2, Cat. No. 553141/553142) be used. If Mouse Fc Block™ is used, it is important that the second step anti-rat IgG antibody does not cross-react with the 2.4g2 mAb (rat IgG2b, κ); we have found that FITC-labeled anti-rat IgG1 mAb RG11/39.4 (Cat. No. 553892) is effective.

## Suggested Companion Products

Catalog Number	Name	Size	Clone
553922	Purified Rat IgG1, κ Isotype Control	0.5 mg	R3-34
553141	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.1 mg	2.4G2
553892	FITC Mouse Anti-Rat IgG1	0.5 mg	RG11/39.4
557399	PE Rat Anti-Mouse CD19	0.1 mg	1D3
557401	PE Hamster Anti-Mouse CD11c	0.1 mg	HL3

## Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to [www.bdbiosciences.com/pharming/en/protocols](http://www.bdbiosciences.com/pharming/en/protocols) for technical protocols.
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. Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer. Since endotoxin may also affect the results of functional studies, we recommend the NA/LE (No Azide/Low Endotoxin) antibody format, if available, for in vitro and in vivo use.

## References

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