Technical Data Sheet

PE-Cy™5 Mouse Anti-Rat CD45RA

Product Information

Material Number:	**557015**
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	OX-33
Immunogen:	Leukocyte common antigen purified from rat splenocytes
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Rat
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The OX-33 antibody reacts with a high-molecular-weight form of CD45 found only on B lymphocytes. CD45 is a member of the Protein Tyrosine Phosphatase (PTP) family: Its intracellular (COOH-terminal) region contains two PTP catalytic domains, and the extracellular region is highly variable due to alternative splicing of exons 4, 5, and 6 (designated A, B, and C, respectively), plus differing levels of glycosylation. The CD45 isoforms detected in the rat are cell type-, maturation-, and activation state-specific. The CD45 isoforms play complex roles in T-cell and B-cell antigen receptor signal transduction.

This antibody is routinely tested by flow cytometric analysis. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.

Preparation and Storage

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

The antibody was conjugated with PE-Cy5 (formerly known as BD Cy-Chrome™) under optimum conditions, and unconjugated antibody and free PE-Cy5 were removed by gel filtration chromatography.

Store undiluted at 4° C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Flow cytometry	Routinely Tested

Recommended Assay Procedure:

We have observed that the staining intensity of OX-33 mAb is reduced after fixation of stained leukocytes (≤ 3 hours with 1% formaldehyde). Therefore, one should not fix the stained cells prior to flow cytometry. We have found that freshly-isolated leukocytes and cell lines may wait for analysis in wash buffer at 4°C, without fixation, for up to 18 hours post-staining without loss of viability. Activated lymphocytes may lose viability rapidly, and data should be collected within 5 hours post-staining. It has been noted that immunofluorescent staining of B cells by OX-33 antibody is especially sensitive to fixation with paraformaldehyde; therefore, fixation of stained cells prior to flow cytometry should be avoided.

PE-Cy5 tandem fluorochromes have been reported to bind some classes of human macrophages and granulocytes via Fc receptors, and PE has been reported to bind to mouse B lymphocytes via Fc receptors. Preincubation of mouse leukocytes with Mouse BD Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2 (Cat. No. 553141/553142) can reduce the non-specific binding of PE-Cy5-conjugated reagents to mouse B cells.

Furthermore, we have observed a distinct type of interaction between PE-Cy5 tandem fluorochromes and the splenocytes of SJL, NOD, and MRL mice: B lymphocytes and some other leukocyte subsets are brightly stained, and Mouse BD Fc Block™ has no significant effect. Therefore, PE-Cy5-conjugated reagents should not be used to stain leukocytes of SJL, NOD, or MRL mice. Reagents conjugated to PE, PerCP, PerCP-Cy5.5, Allophycocyanin (APC), and APC-Cy7 tandem fluorochrome can be used on leukocytes from these mouse strains.

Suggested Companion Products

Catalog Number	Name	Size	Clone
550618	PE-Cy™5 Mouse IgG1 κ Isotype Control	0.1 mg	MOPC-31C

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Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/pharmingen/colors.
4. PE-Cy5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the PE-Cy5 tandem fluorochrome, extra care must be taken when using dual-laser cytometers which may directly excite both PE and Cy5™.
5. PE-Cy5 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by the 488 nm light of an Argon ion laser and serves as an energy donor, coupled to the cyanine dye Cy5™, which acts as an energy acceptor and fluoresces at 670 nm. BD Biosciences Pharmingen has maximized the fluorochrome energy transfer in PE-Cy5, thus maximizing its fluorescence emission intensity, minimizing residual emission from PE, and minimizing lot-to-lot variation.
6. Cy™ is a trademark of Amersham Biosciences Limited.
7. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amershasm Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.
8. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
9. 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.

References

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Woollett GR, Barclay AN, et al. Molecular and antigenic heterogeneity of the rat leukocyte common antigen from thymocytes and T and B lymphocytes.. *Eur J Immunol.* 1985; 15:168-173.(Immunogen: Flow cytometry)