

Product datasheet for **AM39021RP-N**

CD45 (PTPRC) (CD45RB) Mouse Monoclonal Antibody [Clone ID: MT4]

Product data:

Product Type:	Primary Antibodies
Clone Name:	MT4
Applications:	FC, IF
Recommended Dilution:	Anti-CD45RB (clone MT4) can be used in flow cytometry or in immunohistochemistry using cytoslots, frozen or paraffin-embedded tissue sections. Flow cytometry: please see "Protocols" below. Labelled reagent is effectively formulated for direct immunofluorescent staining.
Reactivity:	Human
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Specificity:	Clone MT4 reacts with the 190, 205 and 220 kD isoforms of the cell-surface antigen CD45RB. Variations in CD45RB expression can discriminate between Th1 and Th 2 cells, i.e. CD45RB ^{bright} and CD45RB ^{dim} respectively [8]. CD45RB ^{bright} expression on T cells correlates with higher proliferation and IFN- γ production in comparison to CD45RB ^{dim} expression. 90% of lymphocytes are CD45RB positive. Other isoforms can be detected using anti-CD45RA (clone MB1, cat.no. AM39020), or anti-CD45RC (clone MT2, cat.no. AM39022).
Formulation:	0.01 M sodium phosphate, 0.15 M NaCl, pH 7.3, 0.2% BSA, 0.09% sodium azide Label: PE State: Liquid purified Ig fraction Label: <u>Cat. No. Label EX-max (nm) / EM-max (nm)</u> : AM39021RP-N 488, 532 / 578 AM39021PU-N Pure . /
Concentration:	lot specific
Purification:	Affinity chromatography
Conjugation:	PE
Storage:	Store the antibody undiluted at 2-8°C. Fluorochrome labelled product is photosensitive and should be protected from light.
Stability:	Shelf life: one year from despatch.



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Gene Name:	protein tyrosine phosphatase, receptor type C
Database Link:	Entrez Gene 5788 Human P08575
Background:	<p>The CD45 molecule is also known as the Leukocyte Common Antigen (LCA) or T200 antigen, and is comprised of different glycoproteins ranging from 180-240 kD [1,2]. CD45 is a family of transmembrane protein tyrosine phosphatases critically involved in the regulation of lymphocyte activation signals. Expression of CD45 is found on all hemopoietic cells, e.g. granulocytes, monocytes, macrophages and lymphocytes, except mature erythroid cells. In humans, there is heterogeneous expression of CD45 isoforms (RA, RB, RO, RC) on lymphocyte subpopulations. Detection of the different isoforms can distinguish, for example, between naive T cells and memory T cells, which is of interest in patients with immunodeficiency and autoimmune diseases.</p>
Synonyms:	PTPRC, Leukocyte common antigen, L-CA, T200
Note:	<ol style="list-style-type: none">1. Conjugates with brighter fluorochromes, like PE and APC, will have a greater separation than those with dyes like FITC. When populations overlap, the percentage of positive cells using a selected marker can be affected by the choice of fluorescent label.2. Use of monoclonal antibodies in patient treatment can interfere with antigen target recognition by this reagent. This should be taken into account when samples are analyzed from patients treated in this fashion.3. Reagent data performance is based on EDTA-treated blood. Reagent performance can be affected by the use of other anticoagulants. <p><u>Protocol: Flow cytometry method for use with labelled (FITC, R-PE, APC, PerCP or PerCP-Cy5.5) monoclonal antibodies</u></p> <ol style="list-style-type: none">1. Add 100 µl of EDTA-treated blood (i.e. approx. 10e6 leukocytes) to a 5 ml reagent tube. The content of one tube is sufficient to perform one test.2. Add to each tube 10 µl of labelled monoclonal antibody. (Appropriate mouse Ig isotype control samples should always be included in any labelling study). Vortex the tube to ensure thorough mixing of antibody and cells.3. Incubate the tube for 15 minutes at room temperature in the dark.4. Add 100 µl of a lyse reagent.5. Incubate for 10 minutes at room temperature in the dark.6. Add 2 ml of demineralized water and incubate for 10 minutes in the dark.7. Centrifuge the labeled cell suspension for 2 minutes at 1000 x g.8. Remove the supernatant and resuspend the cells in 200 µl of PBS.9. Analyze by flow cytometry within four hours (alternatively, the cells may be fixed by 0.05% of formaline in buffered saline for analysis the next day. Some antigens are readily destroyed upon fixation and this should be taken into account when using this alternative). <p><u>Flow cytometry method for use with dual and triple combinations</u></p> <ol style="list-style-type: none">1. Add 100 µl of EDTA-treated blood (i.e. approx. 10e6 leukocytes) to a 5 ml reagent tube. The content of one tube is sufficient to perform one test.

For combinations with anti-kappa and/or anti-lambda Ig see **Application note** below.

2. Add to each tube 20 µl of labelled monoclonal antibody combination. (Appropriate mouse Ig isotype control samples should always be included in any labelling study).
3. Vortex the tube to ensure thorough mixing of antibody and cells.
4. Incubate the tube for 15 minutes at room temperature in the dark.
5. Add 100 µl of a lyse reagent and mix immediately.
6. Incubate for 10 minutes at room temperature in the dark.
7. Add 2 ml of demineralized water and incubate for 10 minutes in the dark.
8. Centrifuge the labelled cell suspension for 2 minutes at 1000 x g.
9. Remove the supernatant and resuspend the cells in 200 µl of PBS.
10. Analyze by flow cytometry within four hours (alternatively, the cells may be fixed by 0.05% of formaline in buffered saline for analysis the next day. Some antigens are readily destroyed upon fixation and this should be taken into account when using this alternative).

Application note for anti-kappa and/or anti-lambda Ig combinations

Add 2 ml of PBS containing 0.001% (v/v) Heparin (prewarmed to 37°C) to the cell suspension Vortex, centrifuge (2 min at 300x g) and discard the supernatant.

Repeat this step twice.

Resuspend the pelleted blood cells in 100 µl PBS, pH 7.2, containing 0.001% (v/v) Heparin.

Flow cytometry method for use with purified monoclonal antibodies

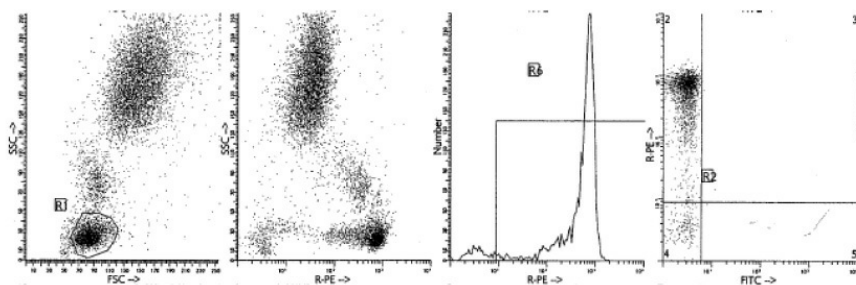
1. Add 100 µl of EDTA-treated blood (i.e. approx. 10e6 leukocytes) to a 5 ml reagent tube. The content of one tube is sufficient to perform one test.
2. Add to each tube 10 µl of purified monoclonal antibody. (Appropriate mouse Ig isotype control samples should always be included in any labelling study). Vortex the tube to ensure thorough mixing of antibody and cells.
3. Incubate the tube for 15 minutes at room temperature in the dark.
4. Wash the labelled cells by adding 2 ml of PBS containing 0.001% (v/v) Heparin, vortexing and centrifuging (2 min 1000 x g) and discard the supernatant.
5. Add 50 µl of appropriate dilution of F(ab)₂ Rabbit Anti Mouse IgG fluorescent conjugate (e.g. FITC or R-PE) in PBS containing 0.001% (v/v) Heparin to the tube. It is recommended that the tube is protected from light.
6. Mix by vortexing and incubate for 15 minutes at room temperature in the dark.
7. Add 100 µl of a lyse reagent and mix immediately.
8. Incubate for 10 minutes at room temperature in the dark.
9. Add 2 ml of demineralized water and incubate for 10 minutes in the dark.
10. Centrifuge the labelled cell suspension for 2 minutes at 1000 x g.
11. Remove the supernatant and resuspend the cells in 200 µl of PBS.
12. Analyze by flow cytometry within four hours (alternatively, the cells may be fixed by 0.05% of formaline in buffered saline for analysis the next day. Some antigens are readily destroyed upon fixation and this should be taken into account when using this alternative).

Protein Families:

Druggable Genome, ES Cell Differentiation/IPS, Phosphatase, Transmembrane

Protein Pathways: Cell adhesion molecules (CAMs), Fc gamma R-mediated phagocytosis, Primary immunodeficiency, T cell receptor signaling pathway

Product images:



Representative Data Flow cytometry analysis of CD45RB monoclonal antibodies is illustrated by flow cytometry analysis of normal blood cells. Direct staining was performed using 10 μ l of PE-conjugated antibody with 100 μ l blood sample.