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Product datasheet for AM39022PU-N

CD45 (PTPRC) (CD45RC) Mouse Monoclonal Antibody [Clone ID: MT2]

Product data:

Product Type:	Primary Antibodies
Clone Name:	MT2
Applications:	FC, IF, IHC
Recommended Dilution:	Anti-CD45RC (clone MT2) can be used in Flow Cytometry or in Immunohistochemistry using cytospots, 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 MT2, specific for CD45RC, reacts with with a 95-115 kD highly sialated glycoprotein. Clone MT2 has previously been described as CD45RA but due to its reactivity with transfectants and its identical staining pattern with ORTH75E4 it is now recognized as CD45RC [7]. Clone MT2 reacts with peripheral blood B cells and is used for the differential diagnosis of non-Hodgkin lymphomas. It also reacts with T suppressor/cytotoxic cells and NK cells. Other isoforms can be detected using anti-CD45RA (clone MB1, cat.no. AM39020), or anti-CD45RB (clone MT4, cat.no. AM39021).
Formulation:	0.01 M Sodium Phosphate, 0.15 M NaCl, pH 7.3 State: Aff - Purified State: Liquid purified Ig fraction Stabilizer: 0.2% BSA Preservative: 0.09% Sodium Azide Label: Cat. No. Label EX-max (nm) / EM-max (nm): AM39022FC-N FITC 488 / 519 AM39022PU-N Pure . /
Concentration:	lot specific
Purification:	Affinity Chromatography
Conjugation:	Unconjugated

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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.

Gene Name: protein tyrosine phosphatase, receptor type C

Database Link: Entrez Gene 5788 Human
P08575

Background: 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.

Synonyms: PTPRC, Leukocyte common antigen, L-CA, T200

Note: 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.

Protocol: Flow cytometry method for use with labelled (FITC, R-PE, APC, PerCP or PerCP-Cy5.5) 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 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).

Flow cytometry method for use with dual and triple combinations
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.

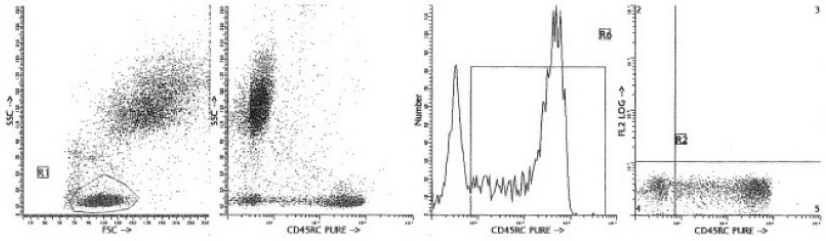
Flow cytometry method for use with purified monoclonal antibodies
1. Add 100 µl of EDTA-treated blood (i.e. approx. 10e6 leukocytes) to a 5 ml reagent tube. The content of one tube is sufficient to perform one test.
2. Add to each tube 10 µl of purified monoclonal antibody. (Appropriate mouse Ig isotype 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 CD45RC monoclonal antibodies is illustrated in the cytogram. Indirect staining was performed by adding 10 µl unlabeled monoclonal antibody to 100 µl blood sample, followed by FITC-labeled secondary antibody.