CD13

Description

Clone WM15
Isotype murine IgG1
Specificity The antibody WM15 recognizes the human CD13 cell surface glycoprotein, a 150kD molecule expressed on granulocytes, endothelial cells, epithelial cells and myeloid progenitors.

Antigen distribution

CD13 antigen is expressed by granulocytes and monocytes and their precursors. Various non-hematopoietic cells express CD13, including epithelial cells from renal proximal tubules and intestinal brush border, endothelial cells, fibroblasts, brain cells, bone marrow stromal cells, osteoclasts and cells lining the biliary caniculae.

Summary

CD13 is a marker for most acute myeloid leukemias and a smaller proportion of acute lymphoid leukemias. CD13 antigen is a zinc-binding metalloprotease which plays a role in cell surface antigen presentation by trimming the N-terminal amino acids from MHC Class II-bound peptides. CD13 ectopeptidase activity is also thought to down-regulate cellular responses to peptide hormones by reducing the local concentration of peptide available for receptor binding. CD13 is upregulated by the anti-inflammatory cytokine IL-4, which suggests a possible indirect mechanism of IL-4 action through the modulation of cell surface antigen processing and/or bioactive peptides. CD13 plays a role in the early events in the interaction between human cytomegalovirus (CMV) and the target cells. CMV incorporates the cellular CD13 protein in its envelope.

Applications

CD13 can be applied in flow cytometry for analysis of blood and bone marrow samples or in immunohistochemistry using cytoprotects or frozen tissue sections or ELISA. CD13 antibodies are used in phenotyping leukemias and also to detect myeloid cells when used together with CD33 antibodies. The antibody inhibits infection of cells by human coronavirus and inhibits aminopeptidase N activity of the CD13 molecule immunoprecipitates.

Usage

All these reagents are effectively formulated for direct immunofluorescent staining of human tissue for flow cytometric analysis using 10 µl/10⁶ leukocytes for singles and 20 µl/10⁶ leukocytes in case of dual and triple combinations. Since applications vary, each investigator should titrate the reagent to obtain optimal results.

Representative Data

Staining with clone WM15 (CD13) monoclonal antibodies is illustrated by flow cytometry analysis of normal blood cells. Direct staining was performed using 10 µl of the R-PE-conjugated antibody with 100 µl blood sample.
Limitations
1. Conjugates with brighter fluorochromes, like PE and APC, will have a greater separation than those with dyes like FITC and CyQ. 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. IQ Products has not characterized the effect of the presence of therapeutic antibodies on the performance of this reagent.
3. Reagents can be used in different combinations, therefore laboratories need to become familiar with the performance characteristics of each antibody in relation with the combined markers in normal and abnormal samples.
4. Reagent data performance is based on EDTA-treated blood. Reagent performance can be affected by the use of other anticoagulants.

Reagents and materials required but not supplied
1. Flow cytometer
2. Flow cytometry disposable 12 x 75-mm capped polystyrene test tubes
3. Micropipette with disposable tips
4. Vortex mixer
5. Centrifuge
6. IQ Lyse - erythrocyte lysing solution (IQP-199)
7. IQ Starflqs – fixation and permeabilization solution (IQP-200)
8. PBS (phosphate-buffered saline)
9. 1% Heparin
10. 1% paraformaldehyde solution in PBS (store at 2-8 °C in amber glass for up to 1 week)

Immunofluorescence staining and lysing protocol
-A- Flow cytometry method for use with purified monoclonal antibodies
1. Add 100 µl of EDTA-treated blood (i.e. approx. 10^6 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*. 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 labeled 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 1:10 dilution of IQ Products F(ab)_2 Rabbit Anti Mouse IgG fluorescent conjugate, [FITC (IQP-190F); R-PE (IQP-190R)] 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 IQ Lyse (IQP-199 ready-to-use) 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 labeled 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).

-B- Flow cytometry method for use with labeled (FITC, R-PE, CyQ, APC, QCD, RQ, FRQ) monoclonal antibodies
1. Add 100 µl of EDTA-treated blood (i.e. approx. 10^6 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 labeled monoclonal antibody*. 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 IQ Lyse (IQP-199 ready-to-use) and mix immediately.
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. 10^6 leukocytes) to a 5 ml reagent tube. The content of one tube is sufficient to perform one test.

2. Add to each tube 20 µl of labeled monoclonal antibody combination*.

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 IQ Lyse (IQP-199 ready-to-use) 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 labeled 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).

* Appropriate mouse Ig isotype control samples should always be included in any labeling study

** PBS: Phosphate Buffered Saline, pH 7.2

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 containing 0.001% (v/v) Heparin

Handling and Storage

Antibodies are supplied either as 100 tests per vial (1 ml) for singles or 50 tests per vial (1 ml) for dual and triple combinations. They are supplied in 0.01 M sodium phosphate, 0.15 M NaCl; pH 7.3, 0.2% BSA, 0.09% sodiumazide (NaN₃). Store the vials at 2-8 °C. Monoclonal antibodies should be protected from prolonged exposure to light. Reagents are stable for the period shown on the vial label when stored properly.

Warranty

Products sold hereunder are warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, which extend beyond the description on the label of the product. IQ Products is not liable for property damage, personal injury, or economic loss caused by the product.

Characterization

To ensure consistently high-quality reagents, each batch of monoclonal antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.

Warning

All products contain sodiumazide. This chemical is poisonous and hazardous. Handling should be done by trained staff only.
References


Explanation of used symbols

Consult instructions for use
Catalogue number
Sufficient for
In Vitro Diagnostic medical device
Caution, consult accompanying document
Keep away from (sun)light
Biological risks
Temperature limitation (°C)
For Research Use Only
Batch code
Use by yyyy-mm-dd
Manufacturer
Authorized Representative in the European Community
Conformité Européenne (European Conformity)

Label - tandem purified material  Ex -max (nm)  Em -max (nm)
P  PURE - -
F  FITC FITC 488 519
R  PE 488, 532 578
C  CyQ PE-Cy5.18 488, 532 667
A  APC 595, 633, 635, 647 660
PC  PerCP 488, 532 678
PCC PerCP-Cy5.5 488, 532 695

IQ Products BV
Rozenburglaan 13a
9727 DL Groningen, The Netherlands
☎ +31 (0)50 57 57 000
☎ +31 (0)50 57 57 002
✉ Technical marketing@iqproducts.nl
✉ Orders orders@iqproducts.nl
✉ www.iqproducts.nl