

PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

CD13

PURE	RUO	REF	IQP-531P	▼	100 tests
FITC	RUO	REF	IQP-531F	▼	100 tests
R-PE	RUO	REF	IQP-531R	▼	100 tests
APC	RUO	REF	IQP-531R	▼	100 tests

RUO
For Research Use Only


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 cytoplots 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.

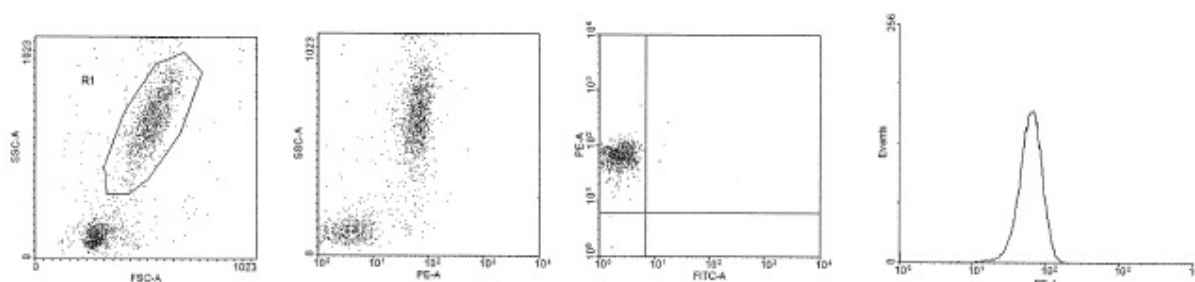
The antibody inhibits infection of cells by human coronavirus and inhibits aminopeptidase N activity of the CD13 molecule immunoprecipitates.

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 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 Starfiqs – 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).

- C - 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.
For combinations with anti-kappa and/or anti-lambda Ig see application note below.
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 (NaN_3). 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

1. Favaloro EJ, Bradstock KF, Kabral A, Grimsley P, Zowtyj H, Zola H.: Further characterization of human myeloid antigens (gp160,95; gp150; gp67): investigation of epitopic heterogeneity and non-haemopoietic distribution using panels of monoclonal antibodies belonging to CD-11b, CD-13 and CD-33. Br J Haematol. 1988 Jun;69(2):163-71.
2. Favaloro EJ.: CD-13 ('gp150'; aminopeptidase-N): co-expression on endothelial and haemopoietic cells with conservation of functional activity. Immunol Cell Biol. 1991 Aug;69 (Pt 4):253-60.
3. Favaloro EJ, Browning T, Nandurkar H.: The hepatobiliary disease marker serum alanine aminopeptidase predominantly comprises an isoform of the haematological myeloid differentiation antigen and leukaemia marker CD-13/gp150. Clin Chim Acta. 1993 Oct 29;220(1):81-90.
4. Bradstock KF, Favaloro EJ, Kabral A, Kerr A, Hughes WG, Berndt MC, Musgrove E.: Human myeloid differentiation antigens identified by monoclonal antibodies: expression on leukemic cells. Pathology. 1985 Jul;17(3):392-9.
5. Bradstock KF, Favaloro EJ, Kabral A, Kerr A, Hughes WG, Musgrove E.: Myeloid progenitor surface antigen identified by monoclonal antibody. Br J Haematol. 1985 Sep;61(1):11-20.
6. Leucocyte Typing III. McMichael A. J. et al (Eds.), Oxford University Press (1987).
7. Leucocyte Typing IV. Knapp W et al. (Eds.), Oxford University Press (1989).
8. Leucocyte Typing V. Schlossman S. et al. (Eds.), Oxford University Press (1995).
9. Favaloro EJ, Browning T, Facey D.: CD13 (GP150; aminopeptidase-N): predominant functional activity in blood is localized to plasma and is not cell-surface associated. Exp Hematol. 1993 Dec;21(13):1695-701.

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	Ex -max (nm)	Em -max (nm)
P	PURE	purified material	-	-
F	FITC	FITC	488	519
R	R-PE	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



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