PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

CD146

PURE	RUO	REF	IQP-560P	100 tests
APC	RUO	REF	IQP-560A	100 tests

RUO ***For Research Use Only***

Description

Clone OJ79c

Isotype murine IgG1

Specificity The antibody (OJ79c) recognizes the 118kD cell surface glycoprotein CD146, also known as MUC18, Mel-CAM and S-endo.

Antigen distribution

CD146 is expressed on endothelial and myeloma cells.

Summary

The expression of CD146 is detected on endothelial cells in vascular tissue throughout the body and is associated with tumor progression and the development of metastasis in human malignant melanoma. The immunoglobulin superfamily member is expressed most strongly on metastatic lesions and advanced primary tumors.
The molecule plays an important role in cell adhesion and cohesion at intercellular junctions in vascular tissue. Because of its expression the molecule allows melanoma cells to interact with cellular elements of the vascular system, leading to the enhancement of hematogeneous tumor spread.

Usage All these reagents are effectively formulated for direct immunofluorescent staining of human tissue for flow cytometric analysis using 10 µl/10^6 cells for singles and 20 µl/10^6 cells in case of dual and triple combinations. Since applications vary, each investigator should titrate the reagent to obtain optimal results.

This antibody (OJ79a) is cross-reactive with pig.

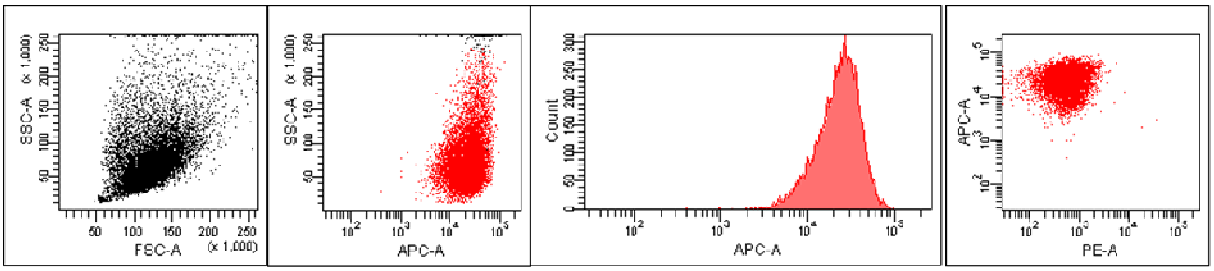
Applications CD146 can be applied in flow cytometry for analysis of blood samples or in immunohistochemistry on frozen sections. It also has been used for ELISA. Reactivity of the antibody used in immunocytochemistry on paraffin slides is unknown.

HLDA Workshop

Leukocyte Typing VI., Kishimoto T. et al. (Eds.), Garland Publishing Inc. (1997).

Representative Data

Staining with CD146 (OJ79c) monoclonal antibodies is illustrated by flow cytometry analysis of Human Umbical Vein Endothelial Cells (HUVECs). Direct staining was performed using 10 µl of the APC-conjugated antibody and 1*10^5 HUVECs.



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. Human Umbical Vein Endothelial Cells (HUVECs)
2. Flow cytometer
3. Flow cytometry disposable 12 x 75-mm capped polystyrene test tubes
4. Micropipette with disposable tips
5. Vortex mixer
6. Centrifuge
7. IQ Lyse - erythrocyte lysing solution (IQP-199)
8. PBS (phosphate-buffered saline) pH 7.2
9. One (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 1* 10^5 Human Umbical Vein Endothelial Cells (HUVECs) in 100 µl PBS 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, etc ...) monoclonal antibodies

1. Add 1*10^5 Human Umbical Vein Endothelial Cells (HUVECs) in 100 µl PBS 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).

** Appropriate mouse Ig isotype control samples should always be included in any labeling study*
*** PBS: Phosphate Buffered Saline, pH 7.2*

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. Kuzu, I. et al. 1993. Lab. Invest. 69: 322-328.
2. Crisan, M. et al. 2008 Cell Stem Cell. 3: 301-13.
3. Park, T.S. et al. 2010 Stem Cells Dev. Oct 5.
4. Leukocyte Typing VI., Kishimoto T. et al. (Eds.), Garland Publishing Inc. (1997).

Explanation of used symbols

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