PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

CD138 FITC / CD38 R-PE

RUO REF IQP-243FR 50 tests

RUO *For Research use only*

Description

CD138 *Clone* **B-A38** *Isotype* **murine IgG**

For detailed description of this particular single reagent, please refer to IQP-153, CD138 (B-A38)

CD38 *Clone* **T16** *Isotype* **murine IgG1**

For detailed description of this particular single reagent, please refer to IQP-132, CD38 (T16)

Intended use

CD138/CD38 dual combination, IQP-243FR, is a direct immunofluorescence reagent used for differentiation in multiple myeloma (MM) samples using flow cytometry.

Summary

CD138 antibodies are used in the detection and monitoring of multiple myeloma, a lympho-proliferative B cell disease, in which malignant plasma cells produce large amounts of immunoglobulins. Rapid and sensitive determination of plasma cell isotype and clonality is carried out by staining for BB4 antigen and cytoplasmic Ig (light and heavy chains).

Another antibody commonly used to detect plasma cells is CD38. However, since CD38 is also expressed on pre-B cells, thymocytes, activated T cells, basophils, natural killer cells, and monocytes it is not a specific plasma cell marker. Antibody B-38 can be helpful in identifying plasma cells in human bone marrow by two color immunofluorescence analysis with antibodies B-A38 and CD38. All cells recognized by monoclonal antibody B-A38 fall within the CD38-bright population. Flow cytometric detection of plasma cells is very sensitive, since as few as 0.5% of plasma cells are detectable in a mononuclear cell population.

Applications

CD38, clone T16, is widely used in flow cytometry to study T cell activation, B cell differentiation and in monitoring immunodeficiency diseases. CD38 is also reactive with multiple myelomas, most cases of ALL (both T and B lineage) and some cases of AML. In immunohistochemistry CD38, clone T16, reacts strongly with plasma cells and cortical thymocytes, less strongly with germinal center B cells. In clinical research CD38 is mainly used for leukemia and lymphoma typing and detection of plasma cells.

CD38 is a type II membrane glycoprotein, with the transmembrane sequence near the N-terminus. Antibodies to human CD38 have a wide range of biological effects, incuding the induction of B and T cell proliferation, protection of B cells from apoptosis, inhibition of B lymphopoiesis and enhancement of macrophage APC function.

Monoclonal antibody B-A38 has been clustered as CD138, and recognizes the syndecan-1 proteoglycan expressed on human plasma cells, endothelial cells and fibroblasts. B-A38 is frequently used for detection of malignant plasma cells in multiple myeloma patients. It does not react with circulating B cells, T cells, monocytes, granulocytes and normal bone marrow cells. In addition, B-A38 is reactive with cytoplasmic Ig+ and surface Ig- plasma cells, indicating a specific reactivity with secreting plasma cells.

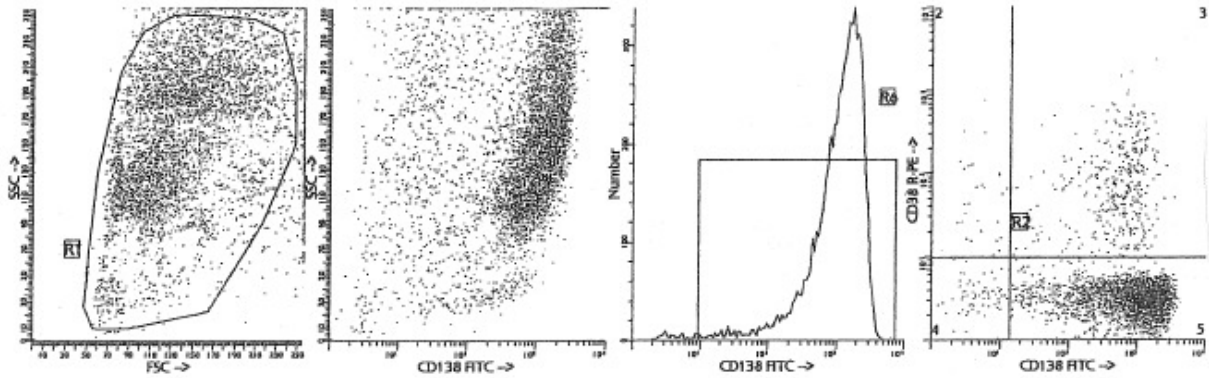
Note: Not all the applications mentioned are performed using IQ Products reagents.

Usage

All these reagents are effectively formulated for direct immunofluorescent staining of human tissue for flow cytometric analysis using 10 µl/10^6 leukocytes for singles and 20 µl/10^6 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 the dual CD138 FITC and CD38 R-PE was performed using 20 µl of the conjugated monoclonal antibody preparation and 100 µl U266 cells.



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

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.

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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