PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

CD138

PURE	RUO	REF	IQP-153P	100 tests	REF	IQP-153P50	50 tests
FITC	IVD	REF	IQP-153F	100 tests	REF	IQP-153F50	50 tests
R-PE	IVD	REF	IQP-153R	100 tests	REF	IQP-153R50	50 tests
CyQ	IVD	REF	IQP-153C	100 tests	REF	IQP-153C50	50 tests
APC	IVD	REF	IQP-153A	100 tests	REF	IQP-153A50	50 tests

RUO ***For Research Use Only***

IVD CE ***In Vitro Diagnostic medical device***

Description

Clone B-A38

Isotype murine IgG1

Specificity CD138 (B-A38) recognizes the 30.5 kD syndecan-1 proteoglycan expressed on human plasma cells, endothelial cells and fibroblasts.

Antigen distribution

CD138 (B-A38) detects 70-100% of multiple myeloma cells and B cell chronic lymphocytic leukaemia.

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.

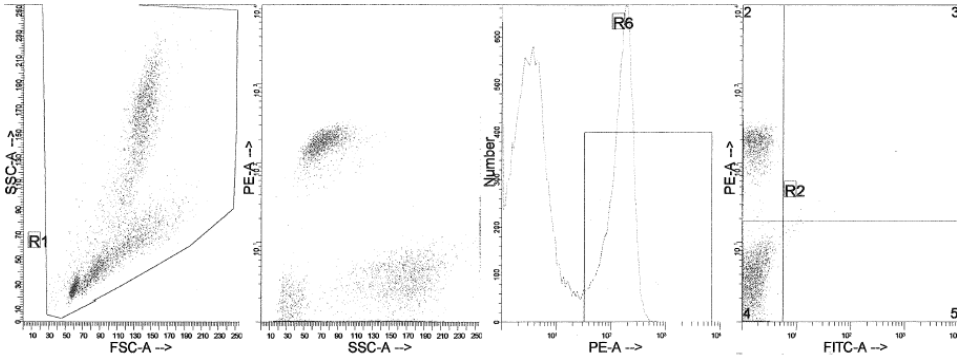
Applications CD138 (B-A38) can be applied in flow cytometry (FCM), immunohistochemistry (IHC) using paraffin embedded tissue sections, western blots and ELISA. IHC application of B-A38 includes reactivity with B cells in lymphoid follicles, plasma cells, epithelium/endothelium in various organs. B-A38 is used in detection and monitoring of multiple myeloma, a lympho-proliferative B cell disease, in which malignant plasma cells produce large amounts of Ig's. Rapid and sensitive determination of plasma cell isotype and clonality is done by B-A38 staining and cytoplasmic Ig (light and heavy chains). Since no reactivity is found with CD34+ precursor cells in bone marrow, B-A38 can be used for bone marrow purging prior to autologous transplantation. CD38 is also used to detect plasma cells, but 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. B-A38 aids in identifying plasma cells in bone marrow by dual FCM with CD38. All cells recognized by B-A38 fall within the CD38-bright population. FCM detection of plasma cells is very sensitive, as few as 0.5% of plasma cells are detectable in a mononuclear cell population.
Syndecan-1 is subject to down modulation from freezing and thawing, trypsinization or allowing the blood sample to stand too long. Re-modulation on vital cells is restored by incubation the cells up to 2 hours at 37 ºC in RPMI. Cell processing should be finished within 6 hours after sample collection as CD138 disappears quite rapidly. The cells can be stored longer by fixation in 1% paraformaldehyde.

HLDA Workshop

6th Workshop on Human Leukocyte Antigens (1996)

Representative Data

Staining with clone B-A38 (CD138) monoclonal antibodies is illustrated by flow cytometry analysis using a patient sample containing plasma cells (Kahler patient). Direct staining was performed using 10 µl of the R-PE-conjugated antibody and 100 µl cells.



Reproducibility

Three different batches of monoclonal antibodies from IQ Products were tested by flow cytometry using a 'lyse-no-wash' method using a patient sample containing plasma cells. Obtained data support the premise that these reagents are equivalent in their reactivity with plasma cells. Values are expressed in terms of % of the total count (see table).

Reagent	Mean % positive	S.D.	% CV	Product code
CD138 FITC	29,39	1,01	3,44	IQP-153F
CD138 R-PE	30,39	0,48	1,58	IQP-153R
CD138 CyQ	29,87	0,38	1,27	IQP-153C
CD138 APC	29,88	1,45	4,85	IQP-153A

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.
5 It is preferable to use these monoclonal antibodies on PBMC fractions of blood or bone marrow samples (see also protocol below***).

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% paraformaldehyde solution in PBS (store at 2-8 °C in amber glass for up to 1 week)
10 Isopaque Ficoll

Immunofluorescence staining and lysing protocol

*** *Isolation of PBMC fraction*

1. Collect 5-10 ml venous blood into heperanized tube or EDTA treated tube using aseptic venipuncture technique.
2. Dilute blood sample 1:1 with PBS (Phosphate Buffered Saline).**
3. Add Ficoll Paque (5 ml) in a centrifuge tube.
4. Carefully layer 5 ml of the diluted blood sample on 5 ml of Ficoll Paque.
5. Centrifuge 600g for 20 minutes.
6. Transfer the PBMC layer to a clean centrifuge tube.
7. Add 5 ml of PBS and centrifuge at 400 g for 15 minutes.
8. Remove the supernatant and add PBS to a concentration of $2.0x10^6$ cells/ml.
9. Continue with procedure flow cytometry method for use with monoclonal antibodies.

- 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 or APC) 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) resp. 50 tests per vial (0.5 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. Zhang et al. 1994. Blood, 83, 3654-3663,
2. Borset et al. 1993. Br. J. Hematol, 85, 446-451
3. Pellat et al..1994. Blood, 84, 2597-2603
4. Van Camp, B. et al. 1990. Blood, 76, 3777
5. Harada, H. et al.. 1993. Blood, 81, 2658
6. Clement, C. et al.. 1995. Leukocyte Typing V, Boston, Eds. S.F. Schlossman et al. Oxford Univ. Press, p714-715
7. Wijdenes, J., et al. 1996. Brit. J. Haematol. 94.318-323
8. Van Zaanen, H.C.T. et al. 1995. Brit. J.Haematol., 91, 55-59
9. Vooijs, W.C., et al. 1996. Cancer Immunol. Immunother. 42. 319-328
10. Ocqueteau, M et al. 1996. Br. J. Haematol. 95,489-493

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)
UK RP	Authorized Representative in the United Kingdom
UK CA	United Kingdom Conformity Assessed
CH REP	Authorized Representative for Switzerland

		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

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