

PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

Anti-MPO

PURE	RUO	REF	IQP-155P	▽	100 tests
APC	RUO	REF	IQP-155A	▽	100 tests
FITC	IVD	REF	IQP-155F	▽	100 tests

RUO IVD **CE** *For Research Use Only*
In Vitro Diagnostic medical device

Description

Clone	266.6K2
Isotype	murine IgG1
Specificity	266.6K2 reacts with the intracellular enzyme MPO.

Antigen distribution

Anti-MPO reacts with the intracellular enzyme, myeloperoxidase in peripheral blood granulocytes. Monocytes are negative or slightly positive while eosinophils and lymphocytes are negative. Anti-MPO stains promyelocytes, myelocytes, metamyelocytes and mature neutrophil granulocytes in bone marrow.

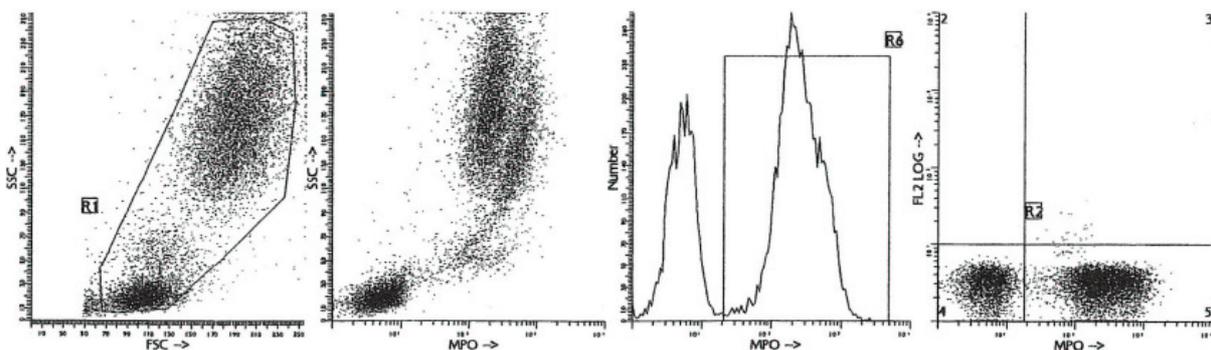
Summary 266.6K2 is used for the diagnosis of AML (acute myeloid leukemia). Non-myeloid leukemia (e.g. acute lymphoblastic leukemia) is usually non-reactive with anti-MPO.

Applications 266.6K2 can be applied in flow cytometry, or in immunohistochemistry using cytopots or frozen tissue sections. The combination of anti-MPO with IQ Starfiqs (IQP-200, for permeabilization and fixation of peripheral blood leukocytes) enables the rapid determination of intracellular MPO by flow cytometry. In a study comparing anti-MPO with other myeloid-reactive monoclonal antibodies such as CD13, CD14, CD15 and CD33, a higher diagnostic sensitivity and specificity was found for AML using anti-MPO. In most patients, more MPO-positive cells were detected by anti-MPO than by cytochemical staining with Sudan black and standard peroxidase methods.

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 266.6K2 (anti MPO intracellular) monoclonal antibodies is illustrated by flow cytometry analysis of normal blood cells. Direct staining was performed using 10 µl FITC-conjugated antibody with 100 µl of blood sample using IQ Starfiqs. Cells were fixed followed by permeabilization and incubation with anti-MPO.



Reproducibility

Monoclonal antibodies from IQ Products were tested by flow cytometry using Starfiqs method on whole blood from healthy donors. Obtained data support the premise that these reagents are equivalent in their reactivity with peripheral blood leukocytes. Values are expressed in terms of % of the total leukocyte count (see table).

Reagent	n	Mean % positive	S.D.	% CV	Product code
anti-MPO FITC	10	64,06	5,07	7,92	IQP-155F

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)

IQ Starfiqs™: intracellular staining using flow cytometry

Protocol for immuno-fluorescence staining of intracellular antigens

IQ Starfiqs™ is a fixation and permeabilization solution intended for preparation of blood leukocytes before flow cytometry analysis of intracellular antigens. **IQ Starfiqs™** is a **ready to use** product, composed of two reagents used sequentially. The composition of both reagents is adjusted to ensure an optimum performance in flow cytometry analysis. Both reagents should be stored at 2-8 °C till the expiration period as indicated.

For optimal intracellular immunostaining and lysing of erythrocytes, **IQ Starfiqs™** should be used following the complete procedure as indicated below (see protocol). **IQ Starfiqs™** enables the detection of intracellular antigens such as CyCD3, CyCD22, TdT and MPO (myeloperoxidase).

In addition, the application of **IQ Starfiqs™** allows the simultaneous detection of cell surface antigens (see extended protocol **IQ Starfiqs™**). It is important to use both reagents and not to mix with other products. **IQ Starfiqs™** is provided as a ready to use product, to minimize hands on time and the easy handling of samples.

Protocol IQ Starfiqs™ (staining of intracellular antigens)

- Add 100 µl EDTA treated whole blood (bone marrow sample, mononuclear cell suspension) to a reagent tube.
- Add 100 µl **IQ Starfiqs™** fixation reagent (Reagent F).
- Incubate for 15 minutes at room temperature.
- Add 4 ml phosphate buffered saline pH 7.3 and centrifuge for 5 minutes at 300xg.
- Remove supernatant and resuspend the cell pellet in 100 µl of **IQ Starfiqs™** permeabilization reagent (Reagent P).
- Add 10 µl of IQ Products antibody conjugate for single reagent or 20 µl of antibody conjugate for dual reagent.
- Incubate for 15 minutes at room temperature.
- Add 4 ml phosphate buffered saline pH 7.3 and centrifuge for 5 minutes at 300xg.
- Remove supernatant and resuspend the cell pellet in 200 µl of phosphate buffered saline.

Extended Protocol IQ Starfiqs™ (staining of cell surface antigens and intracellular antigens)

- Add antibody conjugate to a reagent tube: 10 µl of antibody conjugate for single reagent directed against a cell surface antigen.
- Add 100 µl of EDTA- or Heparin-treated whole blood and mix well.
- Incubate for 15 minutes at room temperature in the dark.
- Add 4 ml phosphate buffered saline pH 7.3 and centrifuge for 5 minutes at 300xg.
- Remove the supernatant.
- Add 100 µl **IQ Starfiqs™** fixation reagent (Reagent F).
- Incubate for 15 minutes at room temperature.
- Add 4 ml phosphate buffered saline pH 7.3 and centrifuge for 5 minutes at 300xg.
- Remove the supernatant and resuspend the cell pellet in 100 µl of **IQ Starfiqs™** permeabilization reagent (Reagent P).
- Add 10 µl of IQ Products antibody conjugate for single reagent directed against an intracellular antigen.
- Incubate for 15 minutes at room temperature.
- Add 4 ml phosphate buffered saline pH 7.3 and centrifuge for 5 minutes at 300xg.
- Remove supernatant and resuspend the cell pellet in 200 µl of phosphate buffered saline.



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

1. Erber, W.M., et al, 1989. Lancet. 1. 761-5
2. Davey, F.R., 1987. Am J. Hematol. 26. 157-66.
3. Majdic, O., et al, 1984. In. J.Cancer. 33. 617-623
4. Van der Schoot, C.E., 1990. Br. J Haematol. 74. 173-178
5. Griffin J.D., et al 1983. Blood. 62. 557-563
6. Sabbath, K.D., J. Clin. Invest. 75. 746-753

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



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

 **Products**
bright fluorescence

