

## PRODUCT INFORMATION SHEET

### Monoclonal antibodies detecting human antigens

**IgG2a** FITC **IgG1** R-PE **IgG1** CyQ IVD REF IQP-426FRC 50 tests

IVD *For In Vitro Diagnostic use*



#### Description

**anti-IgG2a** Clone **MCG2a** Isotype **Mo IgG2a**  
*For detailed description of this particular single reagent, please refer to IQP-192, IgG2a (MCG2a)*

**anti-IgG1** Clone **MCG1** Isotype **Mo IgG1**  
*For detailed description of this particular single reagent, please refer to IQP-191, IgG1 (MCG1)*

**anti-IgG1** Clone **MCG1** Isotype **Mo IgG1**  
*For detailed description of this particular single reagent, please refer to IQP-191, IgG1 (MCG1)*

**Intended use** IQ Products' triple combination IQP-426FRC Isotype Control IgG2a FITC / IgG1 R-PE / IgG1 CyQ is a 3-color direct immuno-fluorescence reagent for use as a negative control in flow cytometric immunophenotyping of erythrocyte-lysed whole blood (LWB). Triple IQP-426FRC is used to estimate the amount of non-antigen-specific antibody binding (non-specific staining) present, particularly that caused by Fc receptors .

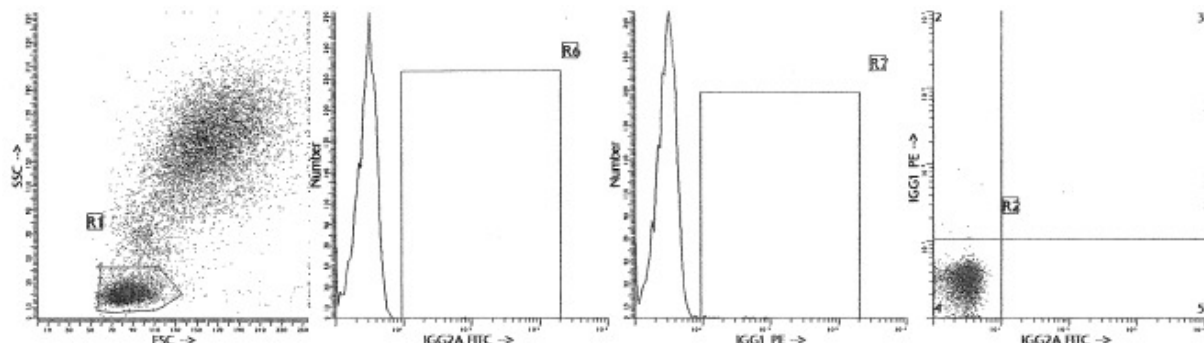
**Summary** IQ Products' Triple combination IQP-426FRC Isotype Control IgG2a FITC/IgG1 R-PE/IgG1 CyQ is used to estimate the amount of non-specific staining.

**Applications** Triple combination IQP-426FRC Isotype Control IgG2a FITC/IgG1 R-PE/IgG1 CyQ contains FITC- and PE-labeled murine monoclonal antibodies that do not bind specifically to antigens on the surface of human leukocytes. However, murine antibodies may bind to human leukocytes by leukocyte Fc receptor interaction with the antibody Fc region. This non-specific binding of monoclonal antibodies can cause difficulty in determining positive reactions. Using the Triple combination IQP-426FRC Isotype Control discrimination between the negative and positive fluorescent populations are established and also defines the background fluorescence as result of this non-specific staining.

**Usage** All these reagents are effectively formulated for direct immunofluorescent staining of human tissue for flow cytometric analysis using 10 µl/10<sup>6</sup> leukocytes for singles and 20 µl/10<sup>6</sup> 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 triple combination IgG2a FITC, IgG1 R-PE and IgG1 CyQ and analysis by flow cytometry is illustrated. Direct staining was performed using 20 µl of the conjugated monoclonal antibody preparation and 100 µl blood sample.



## Reproducibility

Monoclonal antibodies from IQ Products were tested by flow cytometry using IQ Lyse (IQP-199). The used 'lyse-wash' method is on whole blood from healthy donors. Obtained data support the premise that these reagents are equivalent in their reactivity with peripheral blood lymphocytes. Values are expressed in terms of % of the total lymphocyte count (see table).

Reagent	N	Mean % positive	S.D.	Product code
IgG2a FITC	10	0,01	0,02	IQP-426FRC
IgG1 R-PE	10	0,31	0,23	IQP-426FRC
IgG1 CyQ	10	0,38	0,25	IQP-426FRC

## 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 Starfigs - 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<sub>3</sub>). 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. Bakker AB, van den Oudenrijn S, Bakker AQ, Feller N, van Meijer M, Bia JA, Jongeneelen MA, Visser TJ, Bijl N, Geuijen CA, Marissen WE, Radosevic K, Throsby M, Schuurhuis GJ, Ossenkoppele GJ, de Kruif J, Goudsmit J, Kruisbeek AM. C-type lectin-like molecule-1: a novel myeloid cell surface marker associated with acute myeloid leukemia. *Cancer Res.* 2004 Nov 15;64(22):8443-50.

### 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)

		<b>Label - tandem</b>	<b>Ex -max (nm)</b>	<b>Em -max (nm)</b>
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](mailto:marketing@iqproducts.nl)  
Orders [orders@iqproducts.nl](mailto:orders@iqproducts.nl)  
[www.iqproducts.nl](http://www.iqproducts.nl)

