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# PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

**CD15** FITC **CD14** R-PE **CD45** CyQ RUO REF IQP-402FRC 50 tests

RUO ***For Research Use Only***

## Description

**CD15** *Clone* **BRA4-F1** *Isotype* **Mo IgM**

*For detailed description of this particular single reagent, please refer to IQP-129F*

**CD14** *Clone* **UCHM1** *Isotype* **Mo IgG2a**

*For detailed description of this particular single reagent, please refer to IQP-143R*

**CD45** *Clone* **ML2** *Isotype* **Mo IgG1**

*For detailed description of this particular single reagent, please refer to IQP-124C*

## Intended use

IQ Products' triple combination IQP-402FRC CD15 FITC/CD14 R-PE/CD45 CyQ is a 3-color direct immunofluorescence reagent can be used as an extended leukogate. CD45 is a marker present on all human leukocytes. CD14 and CD 15 can be used in the determination of monocytes (CD14) and granulocytes (CD15).

## Applications

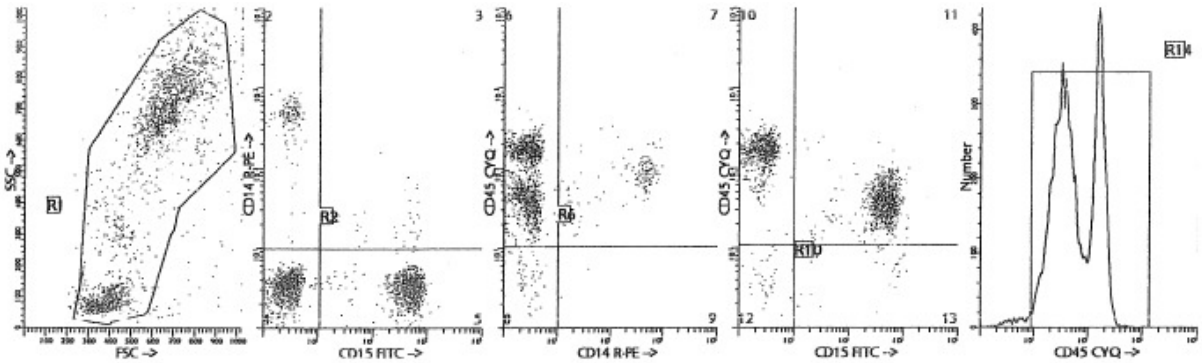
Triple combination IQP-402FRC can be used to make a good separation between granulocytes and monocytes. The expression of CD45 can be used to separate lymphocytes.

## 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 triple combination CD15 FITC, CD14 R-PE and CD45 CyQ analysis was performed using 20 µl of the conjugated monoclonal antibody preparation and 100 µl of blood sample.



**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.

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**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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