

PRODUCT INFORMATION SHEET

Polyclonal antibodies detecting mouse Ig

Polyclonal Rabbit anti-Mouse IgG F(ab')₂

| | | | | | |
|------|---|---|----------|---|-----------|
| FITC | RUO | REF | IQP-190F | ▽ | 100 tests |
| R-PE | RUO | REF | IQP-190R | ▽ | 100 tests |

RUO **For Research Use Only**

Description

Clone Polyclonal

Isotype Rabbit F(ab')₂

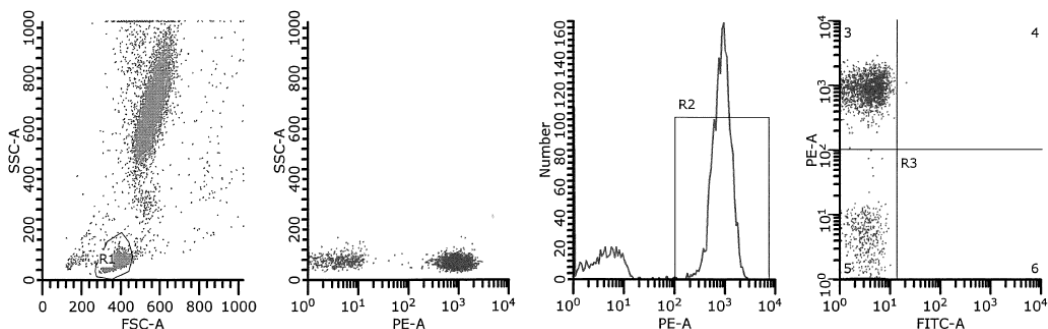
Specificity The antibody reacts with all mouse IgG subclasses (IgG1, IgG2a, IgG2b, IgG3) mouse IgA and mouse IgM, as demonstrated by single radial immunodiffusion using mouse myeloma Ig-proteins isolated from cell lines MOPC21, UPC10, MOPC141, FLOPC21, TEPC15 and TEPC183 respectively. Cross-reaction with human and ox immunoglobulins and fetal calf serum proteins as determined by ELISA is very low, less than 0.5%
The cross-reaction with immunoglobulins of guinea pig, goat, sheep and swine is also low, less than 5%. The cross-reaction with rat immunoglobulins is about 40%

Applications The polyclonal anti-mouse immunoglobulins FITC or R-PE labeled is intended for use in flowcytometry and in immunohistochemistry. The polyclonal has been produced to give optimal performance in flowcytometry when used in combination with a monoclonal mouse antibody. Being the F(ab')₂ fragment of rabbit immunoglobulins, the reagent is particularly well-suited for work on unfixed cells exhibiting Fc receptors, and also for other applications where the Fc part of the antibody molecule could disturb.
The polyclonal antibody is also well-suited for the screening of mouse hybridoma cell cultures for antibody production.

Usage In flowcytometry Rabbit anti mouse immunoglobulins may be used at a volume of 50 µl per test in a dilution of 1:10. (Dilute 0.5 ml polyclonal antibody 10 times in PBS containing 0.001% v/v Heparin and add 50 µl of the diluted sample per test). This is a guideline only.
The optimal conditions may vary depending on specimen and preparation method, and should be determined by each individual laboratory. Note that fluorochrome conjugates are light sensitive, and samples should be protected from light during the staining procedure and until the analysis.

Representative Data

Rabbit anti Mouse immunoglobulins, Staining with polyclonal antibodies is illustrated by flow cytometry analysis of normal blood cells. Indirect staining was performed using 10 µl of human CD3 purified and 50 µl of the polyclonal antibody R-PE conjugated (1:10 diluted) and 100 µl of blood.



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% 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 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)

* 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 (NaN₃). Store the vials at 2-8 °C. Polyclonal 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



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 |



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