PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

anti-IgD FITC RUO REF IQP-509F 100 tests

RUO ***For Research Use Only***

Description

Clone Polyclonal

Isotype Goat F(ab')$_2$

Specificity Anti-IgD recognizes a protein of 50 kD, identified as delta heavy chain of human immunoglobulins.

Antigen distribution

All classes of immunoglobulins have been found on the cell surface of B-lymphocytes where they function as antigen receptors to elicit antigen-dependent proliferation and secretion of antigen specific soluble circulating antibodies.
Anti-IgD (delta) do not cross-react with each other, heavy chains, T cell, monocytes, granulocytes, or erythrocytes.

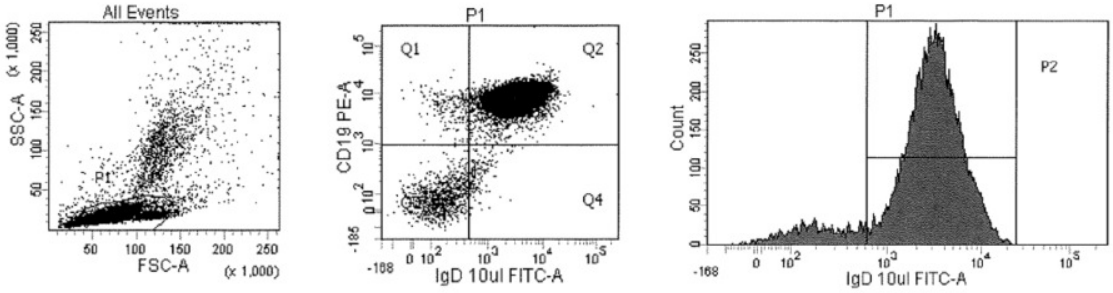
Summary Human immunoglobulins are glycoproteins composed of two disulfide-bonded heavy (H) chain subunits, each of which is linked by interchain disulfide bonds to a light (L) chain forming a tetramolecular complex. There are five classes of immunoglobulins, designated IgG, IgA, IgM, IgD and IgE, which are defined by differences in the constant region of H chains. L chains are divided into kappa or lambda classifications based on structural antigenic differences.
Human surface membrane bound IgM complexes with a hetrodimer of transmembrane proteins of Ig alpha and Ig beta to form the B cell antigen receptor (BCR). The BCR complex plays an important role in B cell development and activation. Transmembrane region of IgM is essential for functional BCR complex assembly.

Applications Anti-IgA, D, G and M are useful in the identification of leukemias, plasmacytomas, and certain non-Hodgkins lymphomas. The most common feature of these malignancies is the restricted expression of a single heavy chain class. Demonstration of clonality in lymphoid infiltrates indicates that the infiltrate is clonal and therefore malignant.

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

Polyclonal antibody, anti-human IgD was analyzed by flow cytometry using a patient sample. Direct staining was performed using 10 µl of the polyclonal antibody and 100 µl of sample.
Note: Detection of intracellular antigens needs an adjusted protocol for immuno staining.



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 Starfiqs – fixation and permeabilization solution (product code IQP-200)
7. PBS (phosphate-buffered saline)
8. 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

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 (immuno-fluorescence 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 (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. De Franco, A.L., et al., 1994, Chem Immunol 59; 156-172
2. Grupp, S.A., et al., 1995, J. Exp. Med. 181:161-168
3. Sites, D.P., et al., 1991, Basic and Clinical Immunology, Appleton & Lange, Norwalk, CT

Explanation of used symbols

- Consult instructions for use
- REF — Catalogue number
- Sufficient for
- 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

		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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