PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

Anti-D BRAD3 APC RUO REF IQP-556A 100 tests

RUO ***For research use only***

Description

Clone BRAD3

Isotype Human IgG3

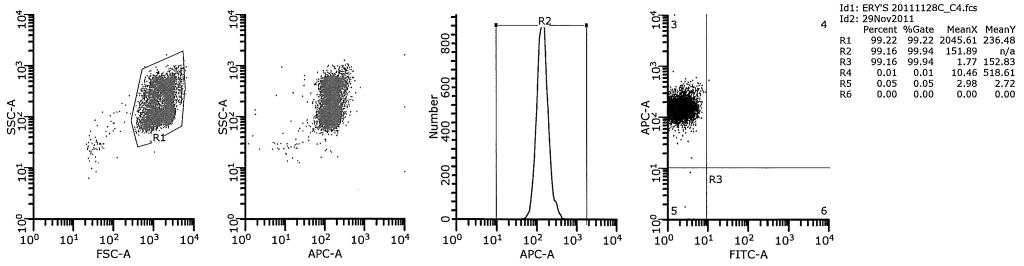
Specificity Clone BRAD3 immunoglobulin (IgG3) is a monoclonal antibody produced by an EBV-transformed B cell line derived from the peripheral blood of an immunized RhD-negative donor. This monoclonal anti-D reacts as an indirect agglutinin with all RhD-positive red cells tested except those of the rare D^{VI} or R_0^{har} types.

Applications Monoclonal antibodies to anti-D, BRAD3, can be applied in flow cytometry for analysis of blood. During pregnancy, fetal blood cells are made in the fetus. The fetal RBCs have the characteristics that are inherited from his/her parents. The mother continues to maintain her own separate blood supply with its own unique characteristics. The placenta acts as a barrier between the blood systems of the mother and her baby and passes oxygen and other nutrients from the mother's blood to that of the child while maintaining the blood supplies separately.
RhD-positive infants born to RhD-negative women may suffer from haemolytic disease of the newborn. The disease can be prevented by administration of anti-D post partum or antenatally. The dosage of anti-D required depends on the size of Feto-Maternal Hemorrhage(FMH). By flow cytometry APC-BRAD3 can be used to quantitate accurately the number of RhD-positive cells in a mixture of RhD-positive and negative cells, and thereby estimate the size of FMH by analysis of a maternal blood sample.

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 BRAD3 clone is illustrated by flow cytometry analysis of normal blood cells. Direct staining was performed using 10 µl of the APC-conjugated antibody and 100 µl red blood cell suspension.



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) pH 7.2
9. 1% paraformaldehyde solution in PBS (store at 2 to 8 °C in amber glass for up to 1 week)

Flow cytometry method use with labeled monoclonal antibodies

1. Collect blood on heparin or EDTA and centrifuge (10 minutes, 600 x g).
2. Wash the pellet of erythrocytes three times with 2 ml of PBS** and centrifuge (2 minutes, 1200 x g).
3. Prepare a 10% erythrocytes suspension in PBS**.
4. Incubate 5 µl of labeled monoclonal* with 10 µl of the 10% erythrocyte suspension for 15 minutes at 37 ºC.
5. Wash twice with PBS** and centrifuge (2 minutes, 1200 x g).
6. Prepare a cell suspension by addition of 500 µl PBS** to the pellet and analyze by flow cytometer.

Note: Appropriate human Ig-isotype control samples should always be included in any labeling study*
*** PBS: Phosphate Buffered Saline, pH 7.2*

References

- The analysis of the results should be according to BCSH Guidelines for FMH which are published in Chapman JF, Working party of the BCSH Blood Transfusion and General Haematology Task Forces (1999) Transfusion Medicine 9, 87-92.
- For NBS centres please refer to the Management Process Description MPD/DDR/RC/034
- Lloyd-Evans *et al*, (1995), Use of a FITC-conjugated monoclonal anti-D (BRAD-3) for quantitation of fetal leaks by flow cytometry, Transfusion Medicine 5, suppl. 1, 23.
- Lloyd-Evans *et al*, (1996), Use of a directly conjugated monoclonal anti-D (BRAD-3) for quantification of fetomaternal hemorrhage by flow cytometry, Transfusion 36, 432-437.
- Lloyd-Evans *et al*, (1999), Detection of weak D and D^{VI} red cells in D-negative mixtures by flow cytometry: implications for feto-maternal haemorrhage quantification and D typing policies for newborns, British J. Haematol. 104, 621-625.

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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.

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	Authorised Representative in the European Community

		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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