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PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

IQ HLA-B27 FITC RUO REF IQP-516F 100 tests

RUO ***For Research Use Only***

Description

Clone ABC-m3 +B7

Isotype Mouse IgG2a

Specificity Com-B27 (Mouse anti HLA-B27 reagent) binds strongly to the HLA-B27 antigen. The Com-B27 formulation demonstrates less cross reactivity to HLA-B7 than other HLA-B27 antibodies. As a negative control the IgG2a isotype control can be used.

Antigen distribution

HLA antigens are products of genes found at the human major histocompatibility complex. These antigens play a central role in the ability of the immune system to differentiate "self" from "non-self" and are essential for communication between cells of the immune system. While HLA antigens are best known for their role in transplantation, certain HLA antigens are associated with specific disease states. One of the best known and strongest associations is between the disease ankylosing spondylitis and the HLA-B27 antigen. 5-10% of the general population is HLA-B27 positive compared to patients with ankylosing spondylitis who are more than 90% positive [1]. In addition, an increased incidence of the HLA-B27 antigen has been reported in patients with Reiter¢s syndrome, anterior uveitis, psoriatic arthritis and inflammatory bowel disease. As a result, tests for the HLA-B27 antigen are a valuable adjunct in the diagnosis of these diseases.

Applications

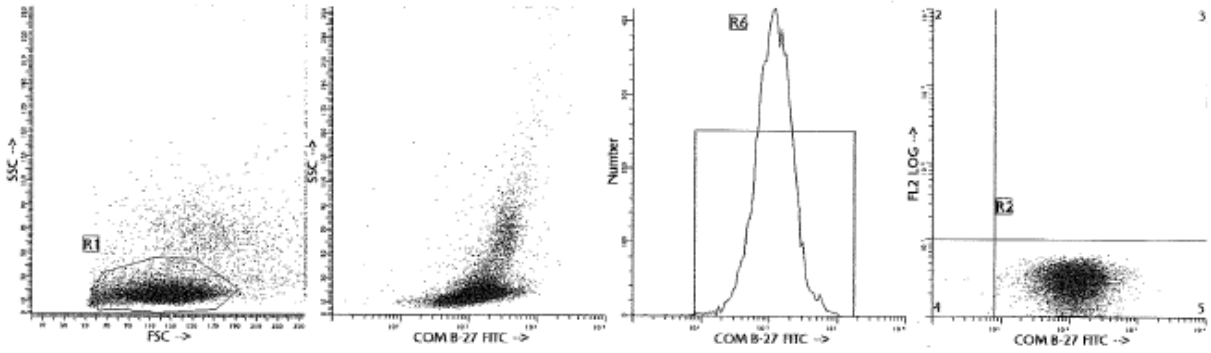
The standard for HLA antigen typing is the microlymphocytotoxicity test described by Terasaki and Mc Clelland [2]. This technique is reliable but requires viable lymphocytes, making sample age, handling and transport critical. Several flow cytometric assays for detection of the HLA-B27 antigen have been described [3-8]. Flow cytometric assays can be performed using small quantities of whole blood without the need to isolate lymphocytes. The specificity is dependent on the anti-HLA-B27 antibody used. Most antibodies give cross-reactivity with HLA-B7, HLA-B22, HLA-B37, and HLA-B39, resulting in false positive results. Janssen [5] reported that HLA-B7 homozygotes could not be differentiated from true HLA-B27 positives. Another problem is that the HLA-B27 antibodies do not detect certain subtypes of HLA-B27. This results in either false positive or false negative results. Therefore, it is very important to use a combination of different HLA-B27 antibodies to minimize the influence of these cross-reactions.
When using Com-B27, a single color reagent containing a special combination of HLA-B27 antibodies for the testing of the presence of HLA-B27 in patient samples, it is possible to discriminate between different cross reactivities and subtypes of HLA-B27 that might occur in patient samples.

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

Com-B27 typing by flow cytometry is performed as a lysed whole blood technique using a single color, directly conjugated antibody combination and gated peripheral blood lymphocytes as the marker population.



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

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 FITC labeled IQ HLA-B27 (IQP-516F)*.
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, 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).

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

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

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3. Albrecht, J., et al., 1987, HLA-B27 typing by use of flow cytofluorometry. Clin Chem; 33(9):1619-1623.
4. Pei R, et al., 1993, A monospecific HLA-B27 fluorescein isothiocyanate-conjugated monoclonal antibody for rapid, simple and accurate
5. HLA-B27 typing. Tissue Antigens; 41(4):200-203.
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8. Zuber, C., et al., 1994, Reliable flow cytometry HLA-B27 typing with B27-FITC/B7-PE combination. Cytometry Supp.:7:77
9. Ward, A.R., et al.,1995, comparison of monoclonal antibodies for flow cytometry analysis of HLA-B2 antigen, cytometry; 22:65-69

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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