PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

CD86

PURE	RUO	REF	IQP-128P	100 tests
FITC	RUO	REF	IQP-128F	100 tests

RUO *For Research Use Only*

Description

Clone BU63

Isotype Murine IgG1

Specificity Clone BU63 produces mouse IgG1 immunoglobulins recognizes the human CD86 surface antigen, also known as B7-2. CD86 antigen is expressed as an 70 kD transmembrane glycoprotein.

Antigen distribution

CD86 is expressed by interdigitating dendritic cells in T zones of secondary lymphoid organs and lower levels by Langerhans cells and peripheral blood dendritic cells. CD86 is expressed by memory B cells and germinal center B cells, centrocytes express more CD86 that centroblasts. Small sIgD+, IgM+ tonsil B cells do not express CD86 but can be induced to express high levels of this molecule on activation in vitro through their surface immunoglobulin, CD40 or MHC class II molecules.
CD86 binds to CD28 and CD152. CD86, like CD80 binds CD28 with a lower avidity than CD152. CD86 binds CD152 with a lower avidity than CD80. CD86 is structurally related to CD80 which was clustered in the Fifth International Workshop on Human Leukocyte Differentiation Antigens.

Summary CD86 acts as one of the ligands for T cell costimulatory molecule CD28. CD86 also binds to CD152, a molecule that has been reported to transduce a negative signal to the T cell (see also CD80). Blockade of CD28 binding to CD86 by CD86 antibodies has been reported to bias the CD28-expressing T cell towards a Th1cell, whereas blockage of CD28 ligation to CD80 polarizes the T cell into a Th2 cell. CD80 and CD86 appear to have similar but no identical effects on cells expressing CD28. CD86 has also been expressed in low levels by monocytes and this expression is increased by culture with IFN-g. Endothelial cells and T cells activated by C3 ligation have also been reported to express CD86 Many T cell clones express both CD80 and CD86. Expression is increased by IL-4 (B-cells) and IFN-g (peripheral blood monocytes) and decreased by IL-10 (peripheral blood dendritic cells).

Applications Monoclonal antibody CD86, clone BU-63, can be applied in flow cytometry.

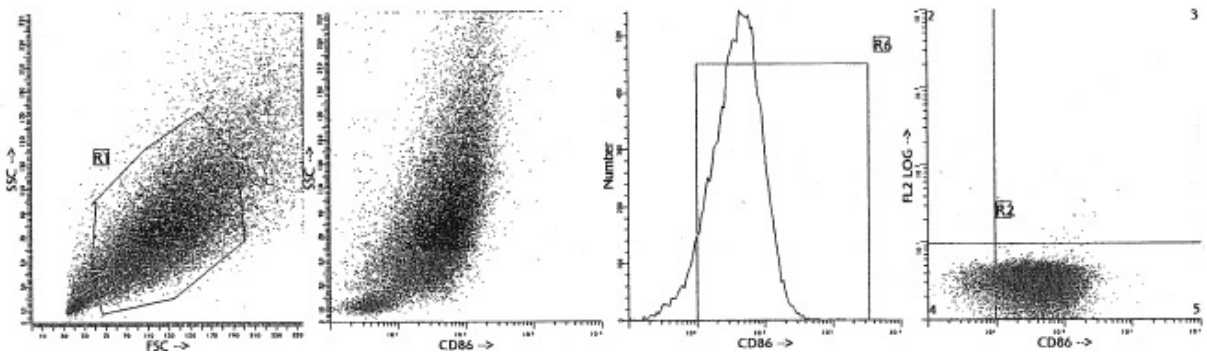
Usage All these reagents are effectively formulated for direct immunofluorescent staining of human tissue for flow cytometric analysis using 10 µL/10^6 leukocytes for singles and 20 µL/10^6 leukocytes in case of dual and triple combinations. Since applications vary, each investigator should titrate the reagent to obtain optimal results.

HLDA Workshop

5th International Workshop on Human Leukocyte Differentiation Antigens, Boston (1993).

Representative Data

Clone Bu-63 (CD86) was analyzed by flow cytometry using human activated lymphocytes which were isolated from a blood sample of a human volunteer. Direct staining was performed using 10 µl of CD86 FITC and 100 µl of lymphocytes.



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

- A - 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)

- B - Flow cytometry method for use with labeled (FITC, R-PE, Cy-Q, APC or PerCP) 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 labeled 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. Add 100 µl of IQ Lyse (IQP-199 ready-to-use) and mix immediately
5. Incubate for 10 minutes at room temperature in the dark
6. Add 2 ml of demineralized water and incubate for 10 minutes in the dark
7. Centrifuge the labeled cell suspension for 2 minutes at 1000 x g
8. Remove the supernatant and resuspend the cells in 200 µl of PBS
9. 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)

- C - 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

References

1. Seino, K., et al., 1995, CD86 on endothelial cells co-stimulates allogeneic CD4+ T cells. Int. Immunol 7 : 1331
2. Kuchroo, V.K., et al, 1995, CD80 and CD86 costimulatory molecules activated differentially the Th1/Th2, Cell 80:707
3. Linsley, P.S., et al., 1994, CD80 and CD86 bind with similar avidities but distinct kinetics to, Immunity 1: 793
4. Prabhu Das, et al., 1995, Reciprocal expression of co-stimulatory molecules, Eur. J. Immunol. 25: 207
5. Ueda, Y., et al., 1995, Both CD28 ligands CD80 and CD86 activate phosphatidylinositol, Int. Immunol. 7: 957
6. Graves, M.F., et al., 1995, In Leukocyte Typing V, Oxford Univ. Press, Oxford

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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