PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

IL-6

PURE	RUO	REF	IQP-164P	50 tests
R-PE	RUO	REF	IQP-164R	50 tests

RUO ***For Research Use Only***

Description

Clone B-E8

Isotype Mouse IgG1

Specificity The antibody B-E8 recognizes human IL-6, also known as 26-kDa protein, B cell stimulatory factor-2 (BSF-2), hybridoma/plasmacytoma growth factor, hepatocyte stimulating factor, cytotoxic T cell differentiation factor, IFN-beta-2 and macrophage-granulocyte inducing factor 2A (MGI-2A).

Antigen distribution

IL-6 is reported to be produced by a large variety of cells like endothelial cells, fibroblasts, activated T cells, hepatocytes, astrocytes, activated monocytes and macrophages.

Summary The immune system reacts to a pathogen by activation of balanced network of the humoral and cellular immune responses. Subsequently the activated condition of the immune system will, after the elimination of the pathogen, be down-regulated to a balanced situation again. Control of the immune response requires efficient communication between the different cells involved in this response. This interaction is provided by cell/cell contact and by a complex array of mediators. Among these mediators cytokines, soluble factors produced by these cells, play an important role.

Cytokines can act on other cells locally or distantly, but can be even auto-regulating. Cytokines can behave stimulatory or inhibitory, or can even perform both activities, depending on the (pre)activation stage of the target cell.[3, 4]

Lymphocytes play an important role in antigen-specific immune responses. Much interest is focusing on the activity of T helper cells. Within this population of white blood cells, cells are defined on the bases of the cytokine pattern they express and actions they take. Two of the extremes in this spectrum of T helper cells are T_H1 cells and T_H2 cells.

T_H1 cells are involved in cell-mediated immune responses, while T_H2 cells have an important role in humoral immune responses. Both T helper cell types express CD3 and CD4 antigens, and no further differentiation can be made immuno-phenotypically. However, functionally, these cells can be distinguished based on the cytokines produced by each subtype.[5, 6, 7, 8] Production of a selected number of cytokines by T cells is illustrated in table 1. Both T_H1 cells and T_H2 cells develop from a mutual precursor the naive T helper cell, which doesn't produce many types of cytokines. Upon stimulation, the right co-stimulation and messengers (like cytokines) the naive T helper cell is skewed into the direction of an effector T helper cell in the spectrum mentioned.

Table 1 - Production of cytokines by human T helper cells type 1 and 2.

Cytokine	*T helper 1 cells*	*T helper 2 cells*
IL-2	++	+
IFN-γ	++	-
TGF-ß	++	-
TNF-α	++	-
IL-4	-	++
IL-5	-	++
IL-13	-	++
IL-3	+/-	+
IL-6	+/-	+
IL-10	+/-	+

Clinically T_H1 and T_H2 cells play an important role in a variety of diseases. As stated above, T_H1 cells appear to be involved in cell-mediated immune-responses like in bacterial infections, development of auto-immune diseases and transplant rejection. Whereas, T_H2 cells appear to be involved in immune protection in response to allergens, but may also lead to allergenic reactivity.[9, 10] Furthermore, development of progressive disease in patients with HIV infection, may be accompanied by a shift from a T_H1 cell response to a T_H2 cell response. These findings can be important for the therapeutic approach of HIV during disease.[11]

Interleukin 6 (IL-6) is a 26 kD protein that is upregulated by cytokines like IL-1, IFN-ß, TNF-α, and IL-17 and down regulated by IL-4 and TGF-ß. IL-6 is reported to be produced by a large variety of cells like fibroblasts, activated T cells, activated monocytes and macrophages. This production occurs after a (systemic) infection and cellular injury. IL-6 is complementary with IL-2 in relation to the proliferation of circulating T lymphocytes and it enhances the effect of IL-3 on multipotential hematopoietic progenitors.

Principles of the Procedure

The level of most of the cytokines produced by immune unstimulated cells is too low to be detected by flow cytometry analysis[19]. After stimulation the level of cytokines is rising and depending on the way of stimulation, the cell population, the secretion inhibitor that is used and several other factors several cytokines are upregulated and in detectable concentrations present. Therefore, a method has been developed to analyze cells which have been stimulated to make detection of these intracellularly expressed cytokines possible.

The by IQ Products developed Cytodetect™ kit (IQP-366) provides reagents for cell stimulation, fixation and permeabilization. The kit also comes with three detailed protocols.

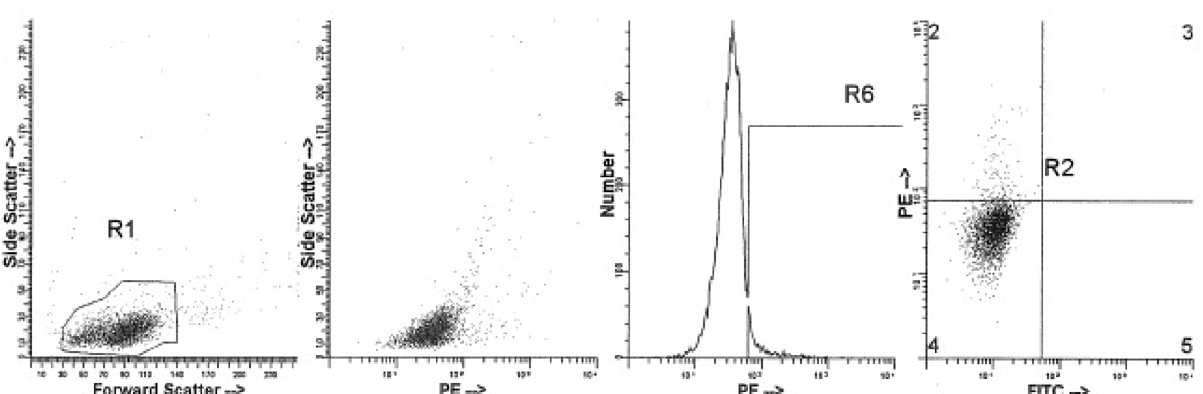
Reagents provided in the Cytodetect™ kit allow processing of 50 blood samples. Each sample can be further analyzed for five (5) different intracellular antigens using one step PE-labeled immuno conjugates, followed by flow cytometry.

Usage

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

Representative Data

Clone B-E8 (anti-IL-6) was analyzed by flowcytometry according to the Cytodetect™ kit protocol. Peripheral blood (lymphocytes) were isolated from a blood sample obtained from a healthy volunteer and subsequently activated, fixed and permeabilized. Direct staining was performed using 10 µl of R-PE-conjugated monoclonal antibody.



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. PBS (phosphate-buffered saline)
7. 1% paraformaldehyde solution in PBS (store at 2-8 °C in amber glass for up to 1 week)

Test Procedure

<u>Note:</u> Unless stated otherwise reagents (including PBS) should be at room temperature when used.

1. *Isolation of blood lymphocytes*

- Collect 5 - 10 ml venous blood into a heparinized tube or EDTA-treated tube, using aseptic venipuncture technique. Process the blood sample within 6 hours of sample collection. In patients with severe neutropenia (absolute neutrophil count less than $200/mm^3$) at least 10 ml of blood may be required.
- Dilute blood sample 1:1 with PBS (Phosphate Buffered Saline).
- Add Ficoll-Paque (5 ml) in a centrifuge tube.
- Carefully layer 5 ml of the diluted blood sample on 5 ml of Ficoll-Paque.
- Centrifuge at 600 g for 20 minutes.
- Transfer the lymphocyte layer to a clean centrifuge tube.
- Add 5 ml of HBSS and centrifuge at 400 g for 15 minutes.
- Remove the supernatant and add 10 ml of RPMI 1640.
- Centrifuge at 300 g for 10 minutes and remove the supernatant.
- Resuspend the cells in RPMI 1640 to a concentration of 2×10^6 cells/ml.
- 1 ml of cell suspension is sufficient for intracellular detection of 5 different cytokines; make sure that besides stimulated cells also unstimulated controls are available.

2. *Stimulation of cells*

- Put 1 ml of the cell suspension into a 24 wells culture plate and add appropriate stimulus and a secretion inhibitor, like Brefeldin A or Monensin. Mix carefully with a pipette.
- Incubate for 5 hours at 37 °C, 5% CO_2.

<u>Note:</u> 5 hours of incubation are suggested in the Cytodetect™ kit. This is an average; the optimal stimulation time has to be determined by the user. The optimum can differ because of the state of cells and kind of cells.

- After stimulation transfer the cells to a centrifuge tube and add 5 ml of HBSS.
- Centrifuge at 300 g for 10 minutes and remove the supernatant.

3. *Fixation of cells*

- Add 500 µl of cold (4 °C) fixative and incubate for 10 minutes at room temperature.
- Add 9 ml of HBSS and centrifuge at 300 g for 10 minutes.
- Remove the supernatant and resuspend the cells in 500 µl of HBSS.
- Cells can be stored overnight at 4 °C or processed further.

4. *Staining of cell surface antigens*

- When cells have been stored overnight in the refrigerator please wash once with 5 ml HBSS. Resuspend the pellet after removing the supernatant in 500 µl of HBSS again.
- Add 10 µl of monoclonal antibody conjugate of choice *against surface* antigen to a 3 ml flow cytometer tube.
- Add 100 µl of cell suspension to the tube and mix well by vortexing, and incubate for 20 minutes at room temperature in the dark.

5. *Permeabilization of cells*

- Add 1.5 ml of permeabilization solution.
- Centrifuge at 200 g for 5 minutes.
- Remove the supernatant and resuspend the cells in 500 µl of permeabilization solution, use 100 µl for staining of intracellular antigens.

6. *Staining of intracellular antigens*

- Add 10 µl of R-PE conjugated monoclonal antibody against intracellular antigens to the reagent tube, and mix well by vortexing.
- Incubate for 20 minutes at 4 °C in the dark.
- Add 1.5 ml of permeabilization solution and centrifuge at 200 g for 5 minutes.
- Remove the supernatant and resuspend the cells in 100 - 300 µl of HBSS.

7. *Analysis by flow cytometry*

- Analyze the cells by flow cytometry.
- Use appropriate controls for immunostaining and data processing.

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Handling and Storage

Antibodies are supplied as 50 tests per vial (0.5 ml). 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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Explanation of used symbols

Symbol	Meaning
	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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