PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

CD25

PURE	RUO	REF	IQP-125P	100 tests	REF	IQP-125P50	50 tests
FITC	IVD	REF	IQP-125F	100 tests	REF	IQP-125F50	50 tests
R-PE	IVD	REF	IQP-125R	100 tests	REF	IQP-125R50	50 tests

RUO ***For Research Use Only***

IVD CE ***In Vitro Diagnostic medical device***

Description

Clone B-B10

Isotype murine IgG1

Specificity CD25 recognizes the 55 kD chain of the interleukin-2 receptor.

Antigen distribution

CD25, otherwise known as the Tac antigen or p55, is the α-chain of the Interleukin-2 (IL-2) receptor. The IL-2 receptor is expressed on activated cells including T cells, B cells and monocytes. It is also present on a subset of thymocytes, HTLV-1 transformed T and B cells, EBV transformed B cells, myeloid precursors and oligodendrocytes. IL-2 induces the expression of the CD25 subunit on NK cells.

Summary

The IL-2 receptor is strongly expressed in Hodgkin's disease, hairy cell leukemia and anaplastic large cell lymphoma. The serum IL-2 receptor is an important parameter in transplantation, inflammatory and malignant disorders.
The functional high affinity IL-2R is composed of a non-covalently associated CD25/CD122/CD132 heterotrimer. The isolated CD25 subunit constitutes a low-affinity IL-2R, while the CD122/CD123 heterodimer binds IL-2 with intermediate affinity. Both the high and intermediate-affinity receptors are important for IL-2 signalling. IL-2 induces the activation and proliferation of T cells, thymocytes, NK cells, B cells and macrophages. Proteolytic cleavage of membrane-bound CD25 generates a soluble form present in human serum. CD25 antibodies are able to inhibit the mixed lymphocyte reaction.

Applications

B-B10 can be applied in flow cytometry for analysis of blood and bone marrow samples, in immunohistochemistry using cytospots or frozen tissue sections or in in vitro assays.
CD25 antibodies are used as a marker of cell activation in transplantation patients, for the detection of cells infected with the human T cell Leukemia viruses I and II, and for the immunophenotyping of lymphomas and leukemias.

Usage

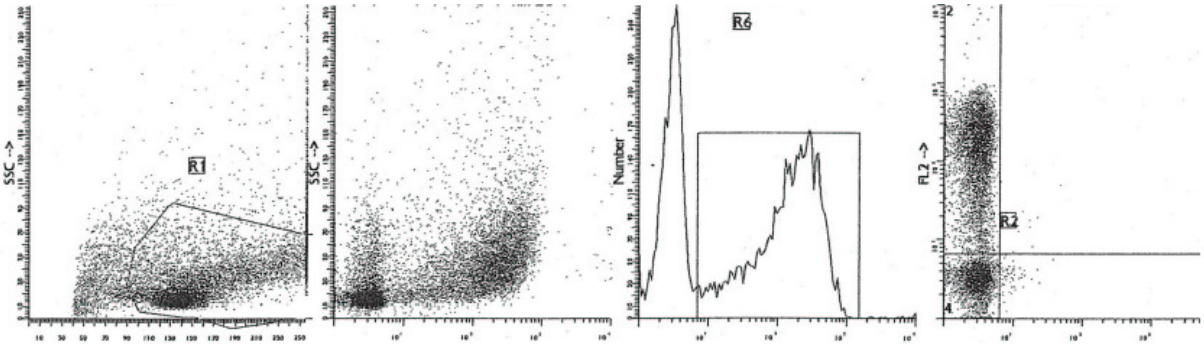
All these reagents are effectively formulated for direct immunofluorescent staining of human tissue for flow cytometric analysis using 10 µl/10^6 leukocytes for singles and 20 µl/10^6 leukocytes in case of dual and triple combinations. Since applications vary, each investigator should titrate the reagent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

HLDA Workshop

4th Leukocyte Typing Workshop - Knapp, W., et al., eds. 1990.

Representative Data

Staining with clone B-B10 (CD25) monoclonal antibodies is illustrated by flow cytometry analysis of activated peripheral blood lymphocytes. Indirect staining was performed using 10 µl of the purified monoclonal antibody with RaM R-PE and 100 µl activated lymphocytes.



Reproducibility

Monoclonal antibodies from IQ Products were tested by flow cytometry using a 'lyse-wash' method on isolated CD3 activated cells from healthy donors. Obtained data support the premise that these reagents are equivalent in their reactivity with peripheral blood lymphocytes. Values are expressed in terms of % of the total lymphocyte count (see table).

Reagent	n	Mean % positive	S.D.	% CV	Product code
CD25 FITC	10	45,64	8,61	18,87	IQP-125F
CD25 R-PE	10	47,86	8,02	16,75	IQP-125R

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, CyQ or APC) 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) resp. 50 test per vial (0.5 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. Barclay, A.N., et al. 1997. The Leukocyte Antigen FactsBook. Academic Press. London
2. Waldman, T.A., 1991 J.Biol.Chem. 266. 2681-2684
3. Callard, R.E., et al., 1994. The Cytokine FactsBook. Academic Prss. London
4. Knapp, W., et al., eds. 1990. Leukocyte Typing IV. Oxford University Press

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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