Miltenyi Biotec

CD25 antibodies

Analyte specific reagents (ASR)

Analytical and performance characteristics were not established

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1. General information

Intended use
4E3 reacts with human CD25. The fluorescently labeled CD25 antigen can be detected by flow cytometry.

Reagents and contents
Monoclonal CD25 antibody conjugates

Product	Volume	REF
CD25-PE	1 mL	170-081-029
CD25-APC	1 mL	170-081-050

2. Technical data and background information

Antigen	CD25
Clone	4E3
Isotype	Murine IgG2b, κ light chain
Alternative names of antigen	IL-2Rα, p55, Tac
Purification	Affinity chromatography
Product formulation	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
+2°C +8°C	Store at +2 °C to +8 °C (+36 °F to +46 °F).
	Store protected from light.
	The use-by date is indicated on the vial label.
	For in-use stability at +2 °C to +8 °C (+36 °F to +46 °F) storage temperature refer to the use-by date indicated on the vial label. Do not use the reagent after the use-by date.

Expression pattern
4E3 recognizes the human CD25 antigen, a 28 kDa glycoprotein, also known as the low-affinity interleukin-2 receptor alpha chain (IL-2Rα). CD25 is expressed on activated T and B cells, on macrophages, and on a subset of non-activated CD4+ regulatory T cells. The CD25 antigen contains three epitope regions called A, B, and C. This CD25 antibody recognizes epitope region B.

3. Warnings and precautions

- Interpretation of results is under the full responsibility of the user.
- For all handling, consideration of good laboratory practice (GLP) regulations is recommended.
- Use of the reagents is restricted to trained and qualified personnel only.
- All biological specimens and all materials that come into contact with blood and blood products must be treated as infectious material. Regulations for the treatment and disposal of infectious material must be followed.
- Reagents contain sodium azide (NaN_3), a chemical highly toxic in pure form. However, at product concentrations, it is not classified as hazardous. Sodium azide may react with lead and copper plumbing to form highly explosive buildups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing. Safety guidelines must be observed.
- For material required but not provided the manufacturers recommendations and safety regulations must be followed.
- Reagents should not be used if signs of leakage are observed. Use undamaged and sealed vials only.

4. Application

Reagents can be used for immunophenotyping by flow cytometry and other research applications.

5. General Use considerations

Principle of method:
The antibody reagent provided enables the identification of a specific target cell type by flow cytometry. This technique is based on fluorochrome conjugated antibodies binding to specific antigens expressed by the target cells. Incubating a sample of interest, e.g., peripheral blood mononuclear cells (PBMC), with the provided antibody reagent leads to fluorescent staining of the cell type expressing the specific target antigen. Analysis of the sample is performed in a flow cytometer at a single-cell level. The analysis is based on the detection of characteristic light emission patterns emitted by the fluorescently labeled antibody upon excitation with laser light. The collected data can be processed and analyzed using flow cytometry software.

Important notes:
Exposure of reagents to temperatures below +2 °C (+36 °F) and above +8 °C (+46 °F) and to light should be minimized during handling.

Sample requirements
- Reagents can be used for determination of antigen-positive cells in whole blood samples by flow cytometry.
- Each cell source can have different storage conditions and limitations that should be considered prior to collection and analysis. For collection of patient samples national legislation must be followed.
- Whole blood samples should be stained within 24 hours.
- Viability of the cells should be assessed and use of samples with at least 80% viable cells is suggested in order to minimize risk of erroneous results.

Quality control:
It is recommended to run regularly a control sample from a normal adult specimen or commercially available whole blood control as a quality control of the system.

6. Analytical specificity

4E3 was tested in the HLDA workshop 4 and proven to specifically recognize human CD25. The results have been published and are referenced in Knapp, W. *et al.* (eds.) 1990, Leukocyte Typing IV Oxford, Oxford University Press.

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7. Excitation and emission data of fluorochrome conjugates

Fluorochrome	Excitation laser (nm)	Excitation maximum (nm)	Emission maximum (nm)
VioBlue®	405	400	452
VioGreen™	405	388	520
VioBright™ FITC	488	496	522
FITC	488	495	520
PE	488 or 561	565	578
PE-Vio®615	488 or 561	565	619
PerCP	488	482	675
PerCP-Vio®700	488	482	676
PE-Vio®770	488 or 561	565	775
APC	561 or 635	652	660
APC-Vio®770	561 or 635	652	775

8. Limitations

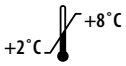
Use of monoclonal antibodies in patient treatment can interfere with recognition of target antigens by this reagent. This should be considered when analyzing samples from patients treated in this fashion. Miltenyi Biotec has not characterized the effect of the presence of therapeutic antibodies on the performance of this reagent.

Reagent data was collected typically with EDTA-treated blood. Reagent performance can be affected by the use of other anticoagulants.

9. References

1. Sakaguchi *et al.* (1995) J. Immunol. 155: 1151-1164. 2. Shevach, E. M. (2001) Certified Professionals: CD4+CD25+ suppressor T cells. J. Exp. Med. 193(11): F41-F46.
3. Maloy, K. J. *et al.* (2001) Regulatory T cells in the control of immune pathology. Nat. Immunol. 2(9): 816-822.
4. Lamprecht, B. *et al.* (2008) Aberrant Blood 112(8): 3339-3347.
5. Chaput, N. *et al.* (2013) Phase I clinical trial combining imatinib mesylate and IL-2: HLA-DR+ NK cell levels correlate with disease outcome. Oncoimmunology 2(2): e23080.
6. Knapp, W. *et al.* (eds.) 1990, Leukocyte Typing IV Oxford, Oxford University Press.

10. Glossary of symbols

	Manufacturer		Use-by date
	Order number		In-use stability
	Batch code		Temperature limit
	Consult instruction for use		Protect from sunlight
	Phone		Website

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