Anti-Human CD99 (3B2/TA8)

	REF	Σ		[A]	
APC	99A2-100T	100 test	20 µg/test	0,05 mg/ml	RUO
PE	99PE2-100T	100 test	20 µg/test	0,05 mg/ml	

1. PRODUCT DESCRIPTION

Clone: 3B2/TA8
Isotype: Mouse IgG2a
Tested application: flow cytometry
Species reactivity: Human
Storage instruction: store in the dark at 2-8 °C
Storage buffer: aqueous buffered solution containing protein stabilizer and 0.09% sodium azide (NaN3).
Recommended usage: Immunostep's CD99, clone 3B2/TA8, is a monoclonal antibody designed for the identification and enumeration of human T cells, plasma cells, and thymocytes that express CD99, utilizing flow cytometry techniques. This reagent is effective for direct immunofluorescence staining of human tissue for flow cytometric analysis using 1 test for 106 cells.
Presentation: liquid
Source: Supernatant proceeding from an in vitro cell culture of a cell hybridoma.
Purification: Affinity chromatography.

2. ANTIGEN DETAILS

Large description: Large description: Anti-CD99 Monoclonal Antibody (Clone 3B2/TA8) targets the CD99 antigen (E2, MIC2), a 32 kDa transmembrane sialoglycoprotein expressed on all leukocyte lineages, particularly high in thymocytes and T cells, while absent in mature granulocytes. This antibody plays a crucial role in T cell adhesion and activation, as well as in hematopoietic adhesion pathways, regulating adhesive properties and programmed cell death distinct from apoptosis. CD99 is differentially expressed during lymphoid and granulocytic development, with significant involvement in T cell differentiation stages, facilitating leukocyte extravasation and spontaneous rosette formation with erythrocytes, independently of PECAM1. Ideal for research in T cell biology and hematopoietic development.(1-4)
Other Names: CD99R, E2, MIC2 gene product, 12E7, E2 antigen, Protein MIC2, T-cell surface glycoprotein E5.
Gene ID: 4267
Molecular weight: CD99 is an approximately 32 kDa sialoglycoprotein and is expressed highly by all leukocytes.

Please, refer to www.immunostep.com technical support for more information.

3. WARRANTY

Warranted only to conform to the quantity and contents stated on the label or in the product labelling at the time of delivery to the customer. Immunostep disclaims hereby other warranties.

Immunostep's sole liability is limited to either the replacement of the products or refund of the purchase price.

4. PROTOCOL

- **Direct Immunofluorescence Cell Surface Staining Protocol**

1. Transfer 100 ul (106 cells/test) of the sample to a 12 x 75 mm polystyrene test tube.
2. Add the suggested volume indicated on the antibody vial to the 12x75 mm cytometer tube.
3. Mix well and incubate in the dark at room temperature at 4 °C for 30 minutes or at room temperature (20-25 °C) for 15 minutes.
4. After the incubation period, add 1,5 ml of an erythrocyte-lysing solution and mix. Incubate at room temperature in the darkness (the blood should be well mixed with the lysing solution).
5. Centrifuge tubes at 540xg for 5 minutes. The supernatant is removed with a Pasteur pipette or with a vacuum pump.
6. Resuspend and wash with 3-5 mL of PBS at 540xg for 5 min.
7. After removing the supernatant and resuspending the cell pellet, add 300 µL of PBS and adquire on the flow cytometer are recorded.
8. Analyse on a flow cytometer or store at 2- 8 °C in the dark until analysis. Samples can be run up to 24 hours after lysis.

- **Indirect Immunofluorescence Cell Surface Staining Protocol**

1. Transfer 100 ul (106 cells/test) of the sample to a 12 x 75 mm polystyrene test tube
2. Add purified reagent according to manufacturer's recommendation and mix gently with a vortex mixer.
3. Incubate in the dark at room temperature at 4 °C for 30 minutes or at room temperature (20-25 °C) for 15 minutes.
4. Add 2 mL 0.01 mol/L PBS (It betters that it containing 2% bovine serum albumin) and resuspend the cells by using a vortex mixer. Centrifuge at 540xg for 5 min in order to remove the McAb not bound to its antigen.
5. Add a secondary conjugated reagent with some fluorochrome and mix. Incubate at room temperature for 15 min in the dark. The absence of light is necessary as the fluorochrome is photoinstability.
6. After the incubation period, add 1,5 ml of an erythrocyte-lysing solution and mix. Incubate at room temperature in the darkness (the blood should be well mixed with the lysing solution). Centrifuge at 540xg for 5 minutes. The supernatant is removed with a Pasteur pipette or with a vacuum pump.
7. Resuspend and a made a final wash with 3-5 mL of PBS at 540xg for 5 min.
8. After removing the supernatant and resuspending the cell pellet, add 300 µL of PBS and adquire on the flow cytometer are recorded.
9. Analyse on a flow cytometer or store at 2- 8 °C in the dark until analysis. Samples can be run up to 24 hours after lysis.

6. REFERENCES

1. Hamilton D, Mallinger R, Millesi H, Engel A, Baumgartner G, Raderer M. Modulation of CD99/MIC2 expression of human AHTO-7 osteoblasts by carcinoma cell line-conditioned media. Anticancer Res2001 Nov-Dec;21(6A):3909-13.

2. Cho NH, Kim YT, Lee JH, Song C, Cho SW, Cho SH, et al. Diagnostic challenge of fetal ontogeny and its application on the ovarian teratomas. Int J Gynecol Pathol2005 Apr;24(2):173-82.

3. Sebire NJ, Gibson S, Rampling D, Williams S, Malone M, Ramsay AD. Immunohistochemical findings in embryonal small round cell tumors with molecular diagnostic confirmation. ApplImmunohistochem Mol Morphol2005 Mar;13(1):1-5.

4. Sokmensuer LK, Muftuoglu S, Asan E. Immunohistochemical analysis of CD71, CD98 and CD99 activation antigens in human palatine and nasopharyngeal tonsils. Saudi Med J2005 Mar;26(3):385-9.

6. EXPLANATION OF SYMBOLS

	Form
REF	Catalog reference
Σ	Contains sufficient for <n> test
	Regulatory Status
	Quantity per test
RUO	Research Use Only
	Manufacturer

7. MANUFACTURED BY:

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