# Anti-Human CD81 (M38)



)	$\bigcirc$	$\sum_{i=1}^{n}$	REF	L.
1 mg/ml		l mg	81PU	PURE
0,01 mg/ml	l µg/test	100 µg	81PU-01MG	PURE
2 mg/ml	20 µL/test	100 test	81F-100T	FITC
2 mg/ml	20 µL/test	100 test	81PE-100T	PE
0,05 mg/ml	5 µL/test	100 test	81A-100T	APC
0,01 mg/ml	l µg/test	100 µg	81B-01MG	Biotin
	0,01 mg/ml 2 mg/ml 2 mg/ml 0,05 mg/ml	1 μg/test 0,01 mg/ml 20 μL/test 2 mg/ml 20 μL/test 2 mg/ml 5 μL/test 0,05 mg/ml	IOO μg I μg/test O,OI mg/ml   IOO test 20 μL/test 2 mg/ml   IOO test 20 μL/test 2 mg/ml   IOO test 20 μL/test 0,05 mg/ml	8IPU 1 mg 1 mg/ml   8IPU-0IMG 100 µg 1 µg/test 0,01 mg/ml   8IF-100T 100 test 20 µL/test 2 mg/ml   8IPE-100T 100 test 20 µL/test 2 mg/ml   8IA-100T 100 test 5 µL/test 0,05 mg/ml

#### 1. PRODUCT DESCRIPTION

Clone: M38;

#### Isotype: IgGI;

Tested application: flow cytometry, western blot;

Immunogen: The anti-CD8I monoclonal antibody derives from MOLT-4 (human T-ALL cell line);

Species reactivity: Human, Feline (cat), Rabbit;

Storage instruction: store in the dark at 2-8 °C;

Storage buffer: aqueous buffered solution containing protein stabilizer and 0.09% sodium azide (NaN<sub>2</sub>);

- Recommended usage: Immunostep's CD8I, clone M38, is a monoclonal antibody intended for:
- Flow cytometry Immunophenotyping: identification and enumeration of TAPA-I. This reagent is effective for direct immunofluorescence staining of human tissue for flow cytometric analysis using I test for 10<sup>6</sup> cells.

Exosomes detection: the products conjugated in PE, FITC, and biotin can be used in combination with #ExoStep Kit and #capture beads. For this application it could be necessary to assay with different quantities.

Western blot: specific exosomes markers are identified with this technique. 1:5000 is the recommended dilution for pure antibodies and 1:500 for biotin antibodies<sup>(4-5)</sup>;



Presentation: liquid;

Source: Supernatant proceeding from an in vitro cell culture of a cell hybridoma; Purification: Affinity chromatography; Other names: TAPA-I, target of an antiproliferative antibody; Gene ID: 975; Molecular weight: 26 kDa.

#### 2. ANTIGEN DETAILS

Large description: This antibody reacts with the CD81-antigen, which is a widely expressed cellsurface protein involved in an astonishing variety of biologic responses. It has been cloned independently several times for different functional effects and is reported to influence adhesion, morphology, activation, proliferation, and differentiation of B, T, and other cells.

On B cells CD81 is part of a complex with CD21, CD19, and Leul3. This complex reduces the threshold for B cell activation via the B cell receptor by bridging Ag specific recognition and CD21-mediated complement recognition. Similarly on T cells CD81 associates with CD4 and CD8 and provides a costimulatory signal with CD3.

CD8I is also physically and functionally associated with several integrins. Anti-CD8I can activate integrin alpha 4 beta 1 (VLA-4) on B cells, facilitating their adhesion to tonsilar interfollicular stroma<sup>(1-3)</sup>.

# WARRANTY

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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. ADDITIONAL INFORMATION

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## 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.
- 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.
- 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 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.
- After removing the supernatant and resuspending the cell pellet, add 300 µL of PBS and adquire on the flow cytometer are recorded.
- 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
- 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.
- 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.
- 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  $\mu L$  of PBS and adquire on the flow cytometer are recorded.
- 9. Analyse on a flow cytometer or store at 2- 8  $^\circ\text{C}$  in the dark until analysis. Samples can be run up to 24 hours after lysis.

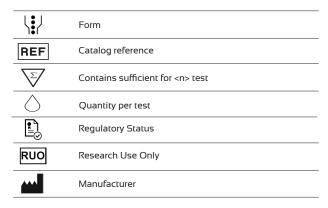
## 6. REFERENCES

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- Sakaguchi N, Kashiwamura S, Kimoto M, Thalmann P, Melchers F. B lymphocyte lineage-restricted expression of mb-1, a gene with CD3-like structural properties. EMBO JI988 Nov;7(11):3457-64.
- Mason DY, Cordell JL, Tse AG, van Dongen JJ, van Noesel CJ, Micklem K, et al. The IgM-associated protein mb-1 as a marker of normal and neoplastic B cells. J Immunoll991 Dec 01;147(11):2474-82.
- Schlossman SF. Leucocyte typing V : white cell differentiation antigens : proceedings of the Fifth International Workshop and Conference : held in Boston, USA, 3-7 November, 1993. Oxford: Oxford University Press; 1995.

# EXPLANATION OF SYMBOLS



# MANUFACTURED BY:



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