Anti-Human CD35 (E11)





PRODUCT DESCRIPTION 1.

Clone: Ell;

Isotype: IgG1; Tested application: flow cytometry;

Immunogen: The anti-CD35 monoclonal antibody derives from human culture cells; 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 (NaN):

Recommended usage: Immunostep's CD35, clone E11, is a monoclonal antibody intended for the identification and enumeration of granulocytes, monocytes, B cells, erythrocytes, follicular dendritic cells, subsets of NK and T cells using flow cytometry. This reagent is effective for direct immunofluorescence staining of human tissue for flow cytometric analysis using I test for 10⁶ cells;

Presentation: liquid:

Source: Supernatant proceeding from an in vitro cell culture of a cell hybridoma; Purification: Affinity chromatography;

Other names: Complement receptor type 1, C3b/C4b receptor, CR1;

Gene ID: 1378:

Molecular weight: 223,63 kDa.

2. ANTIGEN DETAILS

Large description: CD35 is a type I single chain of alvcoprotein, also known as C3b/C4b receptor. Complement Receptor type I or CRI. Four molecular weight allotypes (I60kD, I90kD, 220kD, and 250kD) have been described. CD35 is expressed on granulocytes, monocytes, B cells, erythrocytes, follicular dendritic cells, as well as the subsets of NK and T cells, CD35 binds complement C3b, C4b, or iC3, and iC4, and plays important roles in both innate and adoptive immune response via mediating phagocytosis by granulocytes and monocytes. CD35 has also been reported to inhibit T-cell proliferation.(1, 2)

WARRANTY З.

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.

ADDITIONAL INFORMATION 4.

For research use only. Not for diagnostic use.

Not for resale. Immunostep will not be responsible of violations that may occur with the use of this product. Any use of this product other than the specified in this document is strictly prohibited.

Unless otherwise indicated by Immunostep by written authorization, this product is intended for research only and is not to be used for any other purpose, including without limitation, for human or animal diagnostic, therapeutic or commercial purposes.

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

PROTOCOL

5.

÷. 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 cvtometer 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 $300 \,\mu\text{L}$ 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.

7. EXPLANATION OF SYMBOLS

\ ∎∕	Form
REF	Catalog reference
T	Contains sufficient for > test
\bigcirc	Quantity per test
	Regulatory Status
RUO	Research Use Only
[A]	Concentration
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MANUFACTURED BY:

8.

IMMUNOSTEP S.L.



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REFERENCES

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- 2. 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.