

# Anti-Human C-Myc (9E10)



FITC



235AF-100T



100 test



RUO

## 1. PRODUCT DESCRIPTION

**Clone:** 9E10  
**Isotype:** IgG1  
**Tested application:** flow cytometry  
**Immunogen:** C-terminal region of human c-Myc, aa 408-439  
**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<sub>3</sub>).

**Recommended usage:** Immunostep's c-Myc, clone 9E10, is a monoclonal antibody intended for the identification of c-Myc proto-oncogene using flow cytometry. This reagent is effective for direct immunofluorescence staining of human tissue for flow cytometric analysis using ≤1 µg/10<sup>6</sup> cells.  
**Presentation:** liquid  
**Source:** Supernatant proceeding from an in vitro cell culture of a cell hybridoma.  
**Purification:** Protein A chromatography.

## 2. ANTIGEN DETAILS

**Large description:** The c-myc protein is a 62kD nuclear factor that is ubiquitously expressed in the nucleus. c-myc is part of a heterodimeric complex with MAX that acts as a potent transcriptional activator.

The proto-oncogene c-MYC, has a pivotal function in growth control, differentiation and apoptosis and is among the most frequently affected genes in human cancers (1,2).

The protein encoded by this gene is a multifunctional, nuclear phosphoprotein that plays a role in cell cycle progression, apoptosis and cellular transformation. It functions as a transcription factor that regulates transcription of specific target genes<sup>3</sup>.

**Other Names:** Oncogene Myc, Myc proto-oncogene protein  
**Gene ID:** 4609  
**Molecular weight:** 62 kDa  
Please, refer to [www.immunostep.com](http://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 µl (10<sup>6</sup> 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 acquire 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 µl (10<sup>6</sup> 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 acquire 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.

## 5. REFERENCES

1. Karsten U, Butschak G, Stahn R, Goletz S. A novel series of anti-human glycoprotein A (CD235a) antibodies defining five extra- and intracellular epitopes. *Int Immunopharmacol* Nov;10(11):1354-60.
2. Greaves MF, Sieff C, Edwards PA. Monoclonal antiglycoprotein as a probe for erythroleukemias. *Blood* 1983 Apr;61(4):645-51.
3. Loren MR, Shah VO, Dattilio KL, Civin CI. Flow cytometric analysis of human bone marrow: I. Normal erythroid development. *Blood* 1987 Jan;69(1):255-63.
4. San Miguel JF, Martinez A, Macedo A, Vidriales MB, Lopez-Berges C, Gonzalez M, et al. Immunophenotyping investigation of minimal residual disease is a useful approach for predicting relapse in acute myeloid leukemia patients. *Blood* 1997 Sep 15;90(6):2465-70.
5. Mason D. Leucocyte typing VII : white cell differentiation antigens : proceedings of the Seventh International Workshop and Conference held in Harrogate, United Kingdom. Oxford: Oxford University Press; 2002.

## 6. EXPLANATION OF SYMBOLS



Form



Catalog reference



Contains sufficient for <n> test



Quantity per test



Regulatory Status



Research Use Only



Manufacturer

## 7. MANUFACTURED BY:



**IMMUNOSTEP S.L.**

**Address:** Avda. Universidad de Coimbra, s/n  
Cancer Research Center (C.I.C)  
Campus de Unamuno  
37007 Salamanca (Spain)  
**Tel./fax:** (+34) 923 294 827  
**E-mail:** [info@immunostep.com](mailto:info@immunostep.com)  
[www.immunostep.com](http://www.immunostep.com)