# Anti-Human CD11c (BU/15)

	REF	$\sum$	$\bigcirc$	[A]	
FITC	11CF3-100T	100 test	20 µL/test	2 mg/ml	
PE	11CPE3-100T	100 test	20 µL/test	2 mg/ml	
APC	11CA3-100T	100 test	20 µL/test	2 mg/ml	RUU
PerCP/Cyanine5.5	11CPP5.5-100T	100 test	20 µL/test	2 mg/ml	

## 1. PRODUCT DESCRIPTION

Clone: BU-15:

Isotype: IgG1;

Tested application: flow cytometry;

Immunogen: The anti-CDIIc monoclonal antibody derives from Synovial fluid dendritic 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 CDIIc, clone BU-I5, is a monoclonal antibody intended for the identification and enumeration of human leucocytes using flow cytometry. This reagent is effective for direct immunofluorescence staining of human tissue for flow cytometric analysis using I test for IO<sup>6</sup> cells;

Presentation: liquid;

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

Other names: CR4, pI50, 95, Integrin alpha-X, CDII antigen-like family member C, Leu M5, Leukocyte adhesion glycoprotein pI50,95 alpha chain, Leukocyte adhesion receptor pI50,97; Gene ID: 3687;

Molecular weight: 145 - 150 kDa.

### 2. ANTIGEN DETAILS

Large description: Human CDIIc (alpha X integrin) complexes with CDI8 (beta2 integrin) to form the complement receptor type 4 (CR4) heterodimer which binds to fibrinogen and is involved with monocyte/granulocyte adhesion during inflammatory responses. CDIIc expression is restricted to leukocytes mainly of myeloid lineage with highest expression on macrophages<sup>[1-4]</sup>

#### 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. ADDITIONAL INFORMATION

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.

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## PROTOCOL

5.

- Direct Immunofluorescence Cell Surface Staining Protocol
  - I. Transfer 100 ul (106 cells/test) of the sample to a 12 x 75 mm polystyrene test tube.
  - Add the suggested volume indicated on the antibody vial to the l2x75 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.
  - 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.
  - 7. After removing the supernatant and resuspending the cell pellet, add 300  $\mu L$  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.
- 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 °C in the dark until analysis. Samples can be run up to 24 hours after lysis.

## 6. REFERENCES

- McMichael AJ. Leucocyte typing III: white cell differentiation antigens. Oxford: Oxford University Press; 1987.
- Petty HR, Todd RF, 3rd. Integrins as promiscuous signal transduction devices. Immunol Today1996 May;17(5):209-12.
- Sanchez ML, Almeida J, Vidriales B, Lopez-Berges MC, Garcia-Marcos MA, Moro MJ, et al. Incidence of phenotypic aberrations in a series of 467 patients with B chronic lymphoproliferative disorders: basis for the design of specific four-color stainings to be used for minimal residual disease investigation. Leukemia2002 Aug;16(8):1460-9.
- Sadhu C, Ting HJ, Lipsky B, Hensley K, Garcia-Martinez LF, Simon SI, et al. CDIIc/ CDI8: novel ligands and a role in delayed-type hypersensitivity. J Leukoc Biol2007 Jun;81(6):1395-403.

## 7. EXPLANATION OF SYMBOLS

<b>∖</b> ₽	Form
REF	Catalog reference
$\sum$	Contains sufficient for > test
$\bigcirc$	Quantity per test
	Regulatory Status
RUO	Research Use Only
[A]	Concentration
	Manufacturer

MANUFACTURED BY:

8.

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