Anti-Human HLA-DR (GRB-1)

	REF	Σ		
PURE	HLADRPU	1 mg	1 mg/ml	
FITC	HLADRF-100T	100 test	20 µL/test	2 mg/ml
PE	HLADRPE-100T	100 test	20 µL/test	2 mg/ml
APC	HLADRA-100T	100 test	20 µL/test	2 mg/ml

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1. PRODUCT DESCRIPTION

Clone: GRB-1;
Isotype: IgG1;
Tested application: flow cytometry;
Immunogen: The anti-HLA-DR monoclonal antibody derives from mononuclear cell leukemia acute undifferentiated;
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_3);
Recommended usage: Immunostep's HLA-DR, clone GRB-1, is a monoclonal antibody intended for the identification and enumeration of all human B cells, monocytes and activated T cells using flow cytometry. This reagent is effective for direct immunofluorescence staining of human tissue for flow cytometric analysis using 1 test for 10^6 cells;
Presentation: liquid;
Source: Supernatant proceeding from an in vitro cell culture of a cell hybridoma;
Purification: Affinity chromatography;
Other names: Major Histocompatibility Class II, MHC class II;
Gene ID: 3122;
Molecular weight: 36 kDa.

2. ANTIGEN DETAILS

Large description: This antibody reacts with the HLA-DR-antigen of human B lymphocytes. The antibody reacts with the cells of the monocytic lineage, with myeloblasts and promyelocytes and the cells of B lymphocyte lineage. Polymorphonuclear leukocytes and platelets are found negative[1-6].

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.

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

5. 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. Duraj J, Chorvath B, Sedlak J, Pleskova I. Two-dimensional analysis of metabolically and cell surface radiolabeled proteins of some human lymphoid and myeloid leukemia cell lines. I. 35S-methionine labeled, lactoperoxidase radioiodinated and 3H-reductively methylated proteins. Neoplasma1986;33(5):555-64.
2. Polakova K, Karpatova M. Study of monomorphic determinants on DR molecules of HLA class II antigens. Neoplasma1990;37(3):239-51.
3. Sedlak J, Chorvath B. Fluorescent double labeling of normal and malignant hematopoietic cells by monoclonal antibodies (FITC) and anthracycline cytostatic drug (Daunomycin): a cytometric technique for analysis of drug uptake in hematopoietic cell subpopulations. Neoplasma1991;38(1):13-20.
4. Dusinsky R, Simon M, Ujhazyova J, Polakova K. [Use of monoclonal antibodies against human HLA II antigens for the detection of bovine B lymphocytes and macrophages]. Vet Med (Praha)1992 Sep-Oct;37(9-10):549-54.
5. Mendez R, Serrano A, Jager E, Maleno I, Ruiz-Cabello F, Knuth A, et al. Analysis of HLA class I expression in different metastases from two melanoma patients undergoing peptide immunother apy. Tissue Antigens2001 Jun;57(6):508-19.
6. Paco L, Garcia-Lora AM, Casares C, Cabrera C, Algarra I, Collado A, et al. Total loss of HLA class I expression on a melanoma cell line after growth in nude mice in absence of autologous antitumor immune response. Int J Cancer2007 Nov 01;121(9):2023-30.

7. EXPLANATION OF SYMBOLS

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

8. MANUFACTURED BY:

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