Anti-Human β-2 Microglobulin (GRH1)

	REF	Σ		[A]	
PURE	BETA2PU	1 mg	10 µg/test	1 mg/ml	RUO (GMP)
PURE	beta2PU-01MG	100 test	10 µg/test	1 mg/ml	
PerCP	BETA2PP-100T	100 test	5 µL/test	0,2 mg/ml	
PE	BETA2PE-100T	100 test	20 µL/test	0,05 mg/ml	
PerCP-Cyanine5.5	BETA2PP5.5-100T	100 test	5 µL/test	0,2 mg/ml	

1. PRODUCT DESCRIPTION

Clone: GRH1;
Isotype: IgG1;
Tested application: flow cytometry;
Immunogen: The anti-β-2 microglobulin monoclonal antibody derives from human beta2-microglobulin;
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 β-2 microglobulin, clone GRH1, is a monoclonal antibody intended for the identification and enumeration of B2M protein, a component of the class I major histocompatibility complex (MHC) involved in the presentation of peptide antigens to the immune system 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: β2M, β2-M, beta2-microglobulin;
Gene ID: 567;
Molecular weight: 12 - 14 kDa.

2. ANTIGEN DETAILS

Large description: This antibody reacts with the beta2-microglobulin (B2M) associated with cell-surface MHC Class I molecules and other membrane antigens as well as with soluble B2-microglobulin.

In the immunoprecipitation test the GRH1 two bands were precipitated on SDS-PAGE analysis of 43 kDa and 12 kDa corresponding to the heavy chain of the HLA-A, B and C antigens encoded by a gene on chromosome 6, and the beta 2-microglobulin which is a non-glycosylated protein noncovalently bound to the heavy chain that is encoded by a gene on chromosome 15 (Entrez Gene (human): 15q21-q22.2).[1-6]

3. WARNINGS AND RECOMMENDATIONS

The high expression of b2 microglobulin in leukocytes produces high fluorescence intensity even with low brightness fluorochromes or non-saturating concentrations. This may overlap in other channels and hinders flow cytometer compensation. We recommended adding between 0.5 – 1 mg purified b2 microglobulin to avoid this matter (ref. beta2PU-01MG).

4. 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.

5. ADDITIONAL INFORMATION

For research use only. This reagent has been manufactured and tested in compliance with ISO 13485:2016 Quality Manager System and with relevant Good Manufacturing Practices (GMP) guidelines.

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.

6. 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.

7. REFERENCES

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2. Desoye G, Dohr GA, Motter W, Winter R, Urdl W, Pusch H, et al. Lack of HLA class I and class II antigens on human preimplantation embryos. J Immunol1988 Jun 15;140(12):4157-9.
3. Williams DB, Barber BH, Flavell RA, Allen H. Role of beta 2-microglobulin in the intracellular transport and surface expression of murine class I histocompatibility molecules. J Immunol1989 Apr 15;142(8):2796-806.
4. Danliczyk UG, Delovitch TL. Beta 2-microglobulin induces a conformational change in an MHC class I H chain that occurs intracellularly and is maintained at the cell surface. J Immunol1994 Oct 15;153(8):3533-42.
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6. Perez-Andres M, Almeida J, Martin-Ayuso M, De Las Heras N, Moro MJ, Martin-Nunez G, et al. Soluble and membrane levels of molecules involved in the interaction between clonal plasma cells and the immunological microenvironment in multiple myeloma and their association with the characteristics of the disease. Int J Cancer2009 Jan 15;124(2):367-75

8. EXPLANATION OF SYMBOLS

	Form
REF	Catalog reference
Σ	Contains sufficient for > test
	Quantity per test
	Regulatory Status
RUO	Research Use Only
[A]	Concentration
	Manufacturer

9. MANUFACTURED BY:

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