Isotype Control IgG3 (PPV-07)

	REF	Σ		
PURE	ICIGG3PU-100	0,1 mg	0,1 mg/ml	RUO
FITC	ICIGG3F-100	100 test	20 µL/test	
PE	ICIGG3PE-50	100 test	20 µL/test	
APC	ICIGG3A-50	100 test	20 µL/test	

1. PRODUCT DESCRIPTION

- **Clone:** PPV-07
- **Isotype**: IgG3
- **Tested application**: flow cytometry;
- **Host:** mouse

- **Species reactivity**: Human, mouse and rat;
- **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 isotype control IgG3, clone PPV-07, is a monoclonal antibody produced against a synthetic hapten, which is normally not present in humans or animals using in flow cytometry. This reagent fis useful for screening low background on a variety of mouse and human tissues and is effective for direct immunofluorescence staining of human tissue for flow cytometric analysis using ≤1 µg/106 cells.
- **Presentation**: liquid;
- **Source**: Supernatant proceeding from an in vitro cell culture of a cell hybridoma;
- **Purification**: Affinity chromatography;
- **Storage instruction:** Shipped at ambient conditions, upon arrival store at 4°C.

2. REAGENTS PROVIDED

Mouse anti-human monoclonal isotype control that is sufficient for at 100 assays. This reagent contains 2 ml in PBS and 0,09% NaN3 (sodium azide) as preservative, pH 7,2.

3. REAGENTS NO PROVIDED

Wash solution: 20 Mm NaH2PO4, 150 NaCl, pH 7.2 + 0,09% Sodium azide (NaN3) + 0,5 % bovine serum albumin.
12x75mm Polystyrene Round Bottom Tubes (cytometer tubes).

4. RECOMMENDATION AND WARNINGS

This product contains sodium azide. In acid conditions, it is transformed into hydrazoic acid, a highly toxic compound. Azide compounds must be diluted in running water before being discarded. These conditions are recommended so as to avoid deposits in plumbing, where explosive conditions could develop.

Do not use after expiration date stamped on vial.

For professional use only.
Protect from light

5. ANTIGEN DETAILS

Large description: Today, all techniques used for both diagnosis and research must have negative controls.

This reagent is a mouse IgG3 isotype control to know the level of background on human cells for flow cytometric analysis. Flow Cytometry, these are known as Isotypic Controls. With these it is possible to ensure that results are reliable(I).

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. After the incubation period, add 1,5 ml of an erythrocyte-lysing solution and mix.
4. 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.
5. Resuspend and wash with 3-5 mL of PBS at 540xg for 5 min.
6. Afte removing the supernatant and resuspending the cell pellet, add 300 µL of PBS and adquire on the flow cytometer are recorded.
7. 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.
5. Centrifuge at 540xg for 5 min in order to remove the McAb not bound to its antigen.
6. 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.
7. 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).
8. Centrifuge at 540xg for 5 minutes. The supernatant is removed with a Pasteur pipette or with a vacuum pump.
9. Resuspend and a made a final wash with 3-5 mL of PBS at 540xg for 5 min.
10. After removing the supernatant and resuspending the cell pellet, add 300 µL of PBS and adquire on the flow cytometer are recorded.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. 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.

8. 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.

9. REFERENCES

1. Mitchell J, Sullam PM. Streptococcus mitis phage-encoded adhesins mediate attachment to {alpha}2-8-linked sialic acid residues on platelet membrane gangliosides. Infect Immun2009 Aug;77(8):3485-90.

10. EXPLANATION OF SYMBOLS

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

11. MANUFACTURED BY:

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