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1. Description

Components	1 mL monoclonal Mouse IgG1 isotype control antibodies, conjugated to various dyes.																
	<table border="0"> <tr><td>FITC</td><td>130-092-213</td></tr> <tr><td>PE</td><td>130-092-212</td></tr> <tr><td>APC</td><td>130-092-214</td></tr> <tr><td>VioBlue®</td><td>130-094-670</td></tr> <tr><td>VioGreen™</td><td>130-096-919</td></tr> <tr><td>PerCP</td><td>130-094-968</td></tr> <tr><td>APC-Vio770™</td><td>130-096-653</td></tr> <tr><td>Biotin</td><td>130-093-018</td></tr> </table>	FITC	130-092-213	PE	130-092-212	APC	130-092-214	VioBlue®	130-094-670	VioGreen™	130-096-919	PerCP	130-094-968	APC-Vio770™	130-096-653	Biotin	130-093-018
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VioGreen™	130-096-919																
PerCP	130-094-968																
APC-Vio770™	130-096-653																
Biotin	130-093-018																
	The antibody concentrations are 55 µg/mL (FITC-conjugate), 22 µg/mL (PE-conjugate), 22 µg/mL (APC-conjugate), 55 µg/mL (VioBlue-conjugate), 55 µg/mL (VioGreen-conjugate), 55 µg/mL (PerCP-conjugate), 11 µg/mL (APC-Vio770-conjugate), or 11 µg/mL (Biotin-conjugate).																
Clone	IS5-21F5 (isotype: mouse IgG1).																
Capacity	100 tests or up to 10 ⁹ total cells.																
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.																
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.																

1.1 Background information

The Mouse IgG1 isotype control antibody clone IS5-21F5 is specific for keyhole limpet hemocyanin (KLH). This protein is not expressed on human cells or cell lines. Therefore, the antibody clone IS5-21F5 can be used as a negative control, to distinguish specific from non-specific binding of mouse IgG1 fluorochrome-conjugated antibodies to human cells, e.g. via Fc receptors, or due to interactions of the fluorochrome with the cell surface.

1.2 Applications

- Mouse IgG1 isotype control antibodies are suitable for assessing the level of background staining for flow cytometric cell analysis of human cells.

1.3 Recommended antibody dilution

The recommended antibody dilution for all Mouse IgG1 conjugates is **1:11 for up to 10⁷ cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry. The antibody is suited for staining of formaldehyd-fixed cells.

Cells should be stained prior to fixation, if formaldehyde is used as a fixative.

1.4 Reagent requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS® BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).
 - ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) Anti-Biotin antibodies conjugated to, e.g., APC (# 130-090-856) as secondary antibody reagent in combination with Mouse IgG1-Biotin.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells without fixation.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

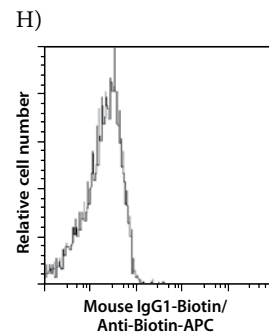
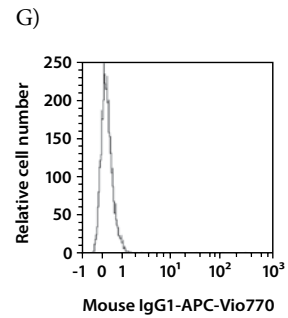
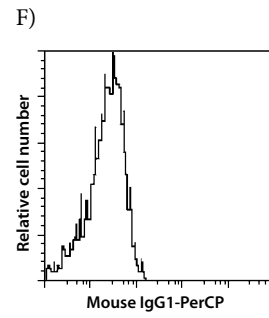
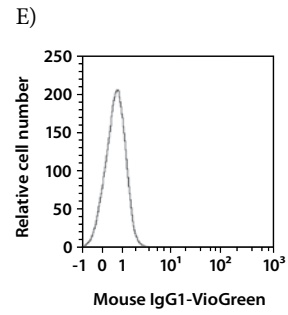
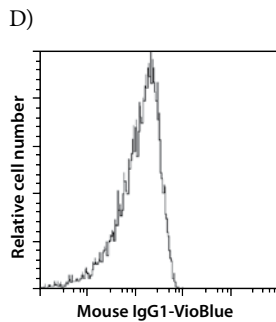
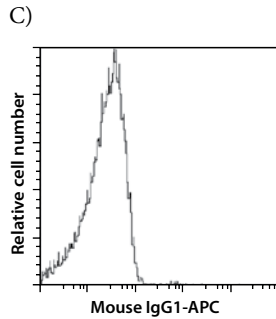
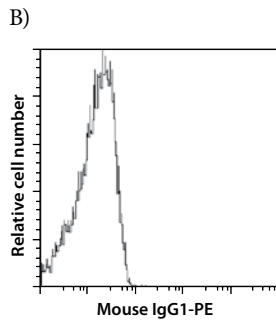
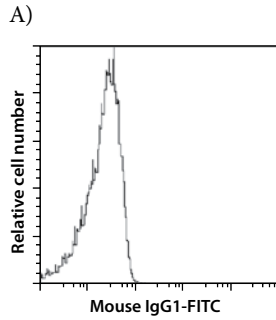
2. General protocol for immunofluorescent staining

▲ Volumes given below are for up to 10⁷ nucleated cells. When working with fewer than 10⁷ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10⁷ nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10⁷ nucleated cells per 100 µL of buffer.
4. Add 10 µL of the Mouse IgG1 isotype control antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
 - ▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Examples of immunofluorescent staining with Mouse IgG1 isotype control antibodies

Human peripheral blood lymphocytes were stained with Mouse IgG1 isotype control antibodies conjugated to FITC (A), PE (B), APC (C), VioBlue (D), VioGreen (E), PerCP (F), or APC-Vio770 (G) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cells stained with Mouse IgG1-Biotin (H) were stained with Anti-Biotin-APC (# 130-090-856). Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence. Cells are gated on viable lymphocytes.



All protocols and data sheets are available at www.miltenyibiotec.com.

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

Warranty

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