



## Antibodies

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### 1. Description

<b>Clone</b>	IS6-11E5.11 (isotype: Mouse IgG2b).
<b>Product format</b>	1 mL Mouse IgG2b antibodies: monoclonal Mouse IgG1 antibodies conjugated to fluorescein-isothiocyanate ( <b>FITC</b> ), R-phycoerythrin ( <b>PE</b> ), allophycocyanin ( <b>APC</b> ) or biotin ( <b>Biotin</b> ). The antibodies are supplied in a solution containing stabilizer and 0.05% sodium azide. The antibody concentrations are 55 µg/mL (FITC-conjugate), 22 µg/mL (PE-conjugate), 22 µg/mL (APC-conjugate), or 11 µg/mL (Biotin-conjugate).
<b>Product size</b>	100 tests (for up to 10 <sup>9</sup> total cells).
<b>Storage</b>	Store protected from light at 4–8 °C. Do not freeze. The expiration date is indicated on the vial label.

#### 1.1 Background and product applications

The Mouse IgG2b isotype control antibody clone IS6-11E5.11 is specific for KLH (keyhole limpet hemocyanin). This protein is not expressed on human cells or cell lines. Therefore, the antibody clone IS6-11E5.11 can be used as a negative control, to distinguish specific from non-specific binding of mouse IgG2b fluorochrome-conjugated antibodies to human cells, e.g. via Fc receptors, or due to interactions of the fluorochrome with the cell surface.

#### Product applications

- Mouse IgG2b isotype control antibodies are suitable for assessing the level of background staining for flow cytometric cell analysis of human cells.

# Mouse IgG2b isotype control antibodies

Mouse IgG2b-FITC	130-092-216
Mouse IgG2b-PE	130-092-215
Mouse IgG2b-APC	130-092-217
Mouse IgG2b-Biotin	130-092-466

### 1.2 Examples of staining concentrations

Mouse IgG2b conjugate	FITC	PE	APC	Biotin
	<b>Recommended antibody dilution</b>			
<b>Flow cytometry<sup>a</sup></b>				
- in general	1:11	1:11	1:11	1:11
- formaldehyde-fixed cells	1:11	1:11	1:11	1:11

a) Given antibody dilutions are for a cell concentration of up to 1×10<sup>8</sup> cells/mL buffer.

### 1.3 Reagent requirements

- Buffer: Prepare a solution containing PBS (phosphate buffered saline) pH 7.2, 0.5% BSA and 2 mM EDTA, e.g. by diluting MACS<sup>®</sup> BSA Stock Solution (# 130-091-376) 1:20 with autoMACS<sup>™</sup> Rinsing Solution (# 130-091-222). Keep buffer cold (4–8 °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 calf serum. Buffers or media containing Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.
- (Optional) PI (propidium iodide) or 7-AAD for flow cytometric exclusion of dead cells without cell fixation. For cell fixation and flow cytometric exclusion of dead cells, the Fixation and Dead Cell Discrimination Kit (# 130-091-163) is recommended.

### 2. General protocol for immunofluorescent staining

▲ Volumes for fluorescent labeling given below are for up to 10<sup>7</sup> nucleated cells. When working with fewer than 10<sup>7</sup> cells, e.g. 10<sup>6</sup> 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 2×10<sup>7</sup> nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Resuspend up to 10<sup>7</sup> nucleated cells per 100 µL of buffer.
2. Add 10 µL of Mouse IgG2b antibodies.
3. Mix well and incubate for 10 minutes in the dark at 4–8 °C.  
▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
4. Wash cells by adding 1–2 mL of buffer per 10<sup>7</sup> cells and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
5. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.
6. If Mouse IgG2b-Biotin was used, resuspend cell pellet in 100 µL buffer, add 10 µL Anti-Biotin antibody (e.g. Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856), and continue as described in step 4–5.

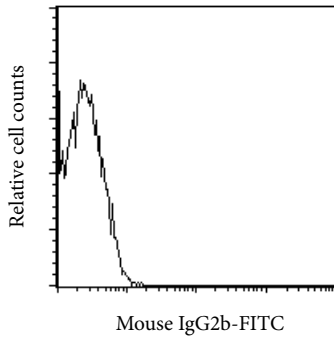
140-001-520/01



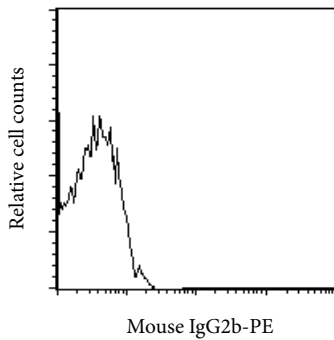
### 3. Examples of staining with Mouse IgG2b antibodies

Human peripheral blood lymphocytes were stained with Mouse IgG2b antibodies conjugated to FITC (a), PE (b), APC (c), or biotin (d) and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

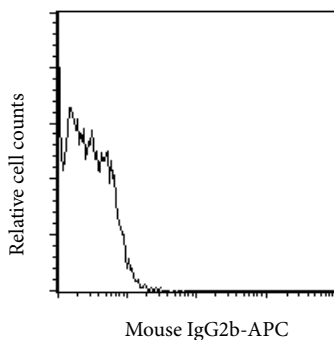
(a) Human peripheral blood lymphocytes stained with Mouse IgG2b-FITC.



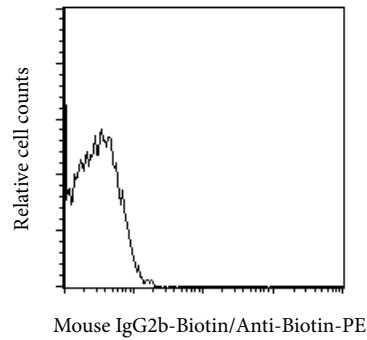
(b) Human peripheral blood lymphocytes stained with Mouse IgG2b-PE.



(c) Human peripheral blood lymphocytes stained with Mouse IgG2b-APC.



(d) Human peripheral blood lymphocytes stained with Mouse IgG2b-Biotin.



#### 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.

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