



# Anti-Ig $\kappa$ light chain antibodies human

Anti-Ig $\kappa$ light chain-FITC	130-093-053
Anti-Ig $\kappa$ light chain-PE	130-093-054
Anti-Ig $\kappa$ light chain-APC	130-093-043
Anti-Ig $\kappa$ light chain-Biotin	130-093-042
Anti-Ig $\kappa$ light chain pure	130-093-041

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## 1. Description

<b>Components</b>	1 mL Anti-Ig $\kappa$ light chain antibodies, human: monoclonal Anti-Ig $\kappa$ light chain antibodies conjugated to fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), allophycocyanin (APC), or biotin. The unconjugated (pure) antibody is supplied at a concentration of 100 $\mu$ g/mL.
<b>Clone</b>	IS11-24D5 (isotype: mouse IgG1).
<b>Capacity</b>	100 tests or up to $10^9$ total cells.
<b>Product format</b>	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
<b>Storage</b>	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

### 1.1 Background information

Immunoglobulins produced by B cells are composed of two heavy and two light chains, connected by disulfide bonds. Differences in the composition of the heavy chain constant regions determine the different isotypes. The light chains of all isotypes are either of  $\kappa$  or  $\lambda$  type. Clone IS11-24D5 detects human Ig light chains of  $\kappa$  type. Heavy chains and light chains of the  $\lambda$  type are not detected.

### 1.2 Applications

- Identification of B cells expressing immunoglobulins of the  $\kappa$  light chain type by flow cytometry or fluorescence microscopy.
- Staining of intracellular immunoglobulins of the  $\kappa$  light chain type in B cells or plasma cells

### 1.3 Recommended antibody dilution

For antibody labeling of human cells.

Anti-Ig $\kappa$ light chain conjugate	FITC	PE	APC	Biotin
<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  $10^7$  cells/ $100 \mu$ L of buffer.

### 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. Buffers or media containing  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  are not recommended for use.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with Anti-Ig  $\kappa$  light chain-Biotin.
- (Optional) Inside Stain Kit (# 130-090-477) for intracellular staining.
- (Optional) Anti-FITC MicroBeads (# 130-048-701), Anti-PE MicroBeads (# 130-048-801), Anti-APC MicroBeads (# 130-090-855), or Anti-Biotin MicroBeads (# 130-090-485).
- (Optional) CD19-FITC (# 130-091-328), CD19-PE (# 130-091-247), CD19-APC (# 130-091-248).
- (Optional) Propidium iodide (PI) 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.

140-002-004-01



## 2. General protocol for immunofluorescent staining

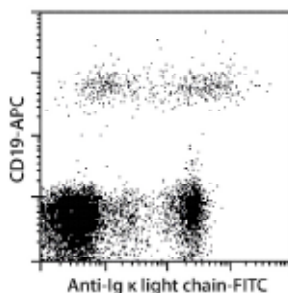
▲ Volumes given below are for up to  $10^7$  nucleated cells. When working with fewer than  $10^7$  cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes, accordingly (e.g. for  $2 \times 10^7$  nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to  $10^7$  nucleated cells per 100  $\mu\text{L}$  of buffer.
4. Add 10  $\mu\text{L}$  of the Anti-Ig  $\kappa$  light chain antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator ( $2-8^\circ\text{C}$ ).  
▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
6. Wash cells by adding 1–2 mL of buffer per  $10^7$  cells and centrifuge at  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
7. (Optional) If Anti-Ig  $\kappa$  light chain-Biotin was used, resuspend the cell pellet in 100  $\mu\text{L}$  of buffer, add 10  $\mu\text{L}$  of anti-biotin antibody (Anti-Biotin-FITC, Anti-Biotin-PE, or Anti-Biotin-APC), and continue as described in steps 5 and 6.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

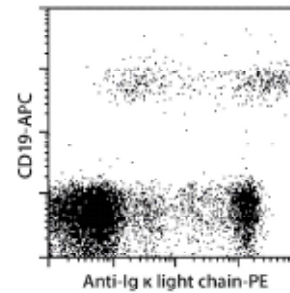
## 3. Examples of immunofluorescent staining with Anti-Ig $\kappa$ light chain antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with Anti-Ig  $\kappa$  light chain antibodies conjugated to FITC (a), PE (b), or APC (c), and analyzed by flow cytometry. Anti-Ig  $\kappa$  light chain-FITC, -PE, and -APC were additionally stained with CD19-PE or CD19-APC. Cells stained with Anti-Ig  $\kappa$  light chain-Biotin (d) were stained with Anti-Biotin-PE (# 130-090-756) as well as CD19-APC. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

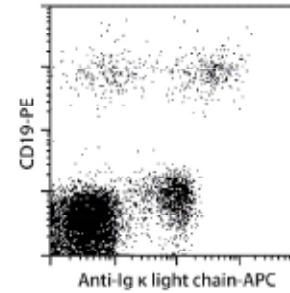
(a) Human PBMCs stained with Anti-Ig  $\kappa$  light chain-FITC and CD19-APC.



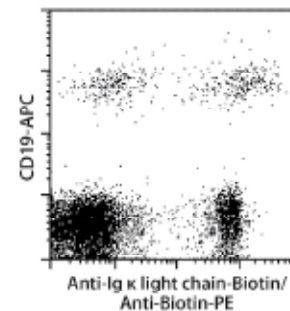
(b) Human PBMCs stained with Anti-Ig  $\kappa$  light chain-PE and CD19-APC.



(c) Human PBMCs stained with Anti-Ig  $\kappa$  light chain-APC and CD19-PE.



(d) Human PBMCs stained with Anti-Ig  $\kappa$  light chain-Biotin, Anti-Biotin-PE, and CD19-APC.



All protocols and data sheets are available at [www.miltenyibiotec.com](http://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.

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