

Anti-H-2K ^k -FITC	130-085-101
Anti-H-2K ^k -PE	130-094-867
Anti-H-2K ^k -APC	130-094-873

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1. Description

Components	1 mL Anti-H-2K ^k antibodies, mouse: monoclonal Anti-H-2K ^k antibodies conjugated to fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), or allophycocyanin (APC).
Clone	H100-27.R55 (isotype: mouse IgG2a).
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 H-2K^k antigen is expressed on mouse MHC I positive cells of H-2K^k haplotype and cells transfected with pMACS K^k.II using MACSelect™ K^k – Transfected Cell Selection Kits.

H-2K^k-FITC is suitable for the direct immunofluorescent staining of H-2K^k positive cells. To control the efficiency of transfection with pMACS K^k, transfected cells are fluorescently stained with Anti-H-2K^k antibodies and analyzed by flow cytometry or fluorescence microscopy.

1.2 Applications

- Identification and enumeration of mouse MHC class I⁺ cells of H-2K^k haplotype by direct immunofluorescence.
- Evaluation of cells transfected with pMACS K^k using MACSelect K^k – Transfected Cell Selection Kit (# 130-091-986).

1.3 Recommended antibody dilution

For antibody labeling of mouse cells.

Anti-H-2K ^k conjugate	FITC	PE	APC
Flow cytometry ^a			
- In general	1:11	1:11	1:11

a) The indicated antibody dilutions are for up to 10⁷ cells/100 μL 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 mouse serum albumin, mouse serum, or fetal bovine serum. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) Mouse IgG2a-FITC (# 130-091-837), Mouse IgG2a-PE (# 130-091-835), or Mouse IgG2a-APC (# 130-091-836) for isotype control.
- (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).

2.1 General protocol for immunofluorescent staining

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 Anti-H-2K^k antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

- 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.
- Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

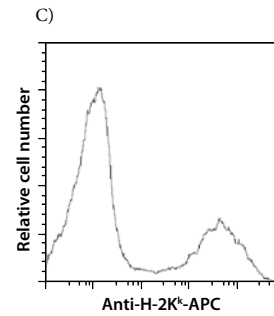
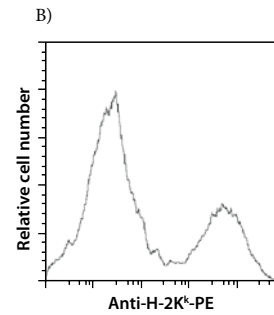
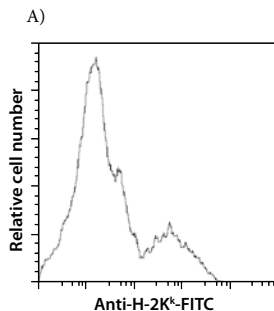
2.2 Immunofluorescent staining of transfected cells

- Perform transfection and culture the transfected cells for an appropriate time. For details refer to the MACSelect K^k – Transfected Cell Selection Kit (# 130-091-986) user manual. To determine the time point of highest H-2K^k expression, stain aliquots of the transfected cells taken after various culture periods.
- Centrifuge the transfected cells at $50\text{--}200\times g$ for 5 minutes at room temperature. Aspirate supernatant completely.
- Resuspend up to 10^7 cells in 100 μL of buffer.
- Add 10 μL of the Anti-H-2K^k antibody.
- Mix well and incubate for 5–10 minutes in the dark in the refrigerator ($2\text{--}8^\circ\text{C}$).
- Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at $50\text{--}200\times g$ for 5 minutes at room temperature.
- For **flow cytometry**: Resuspend cell pellet in appropriate amount of buffer, e.g. 500 μL , and proceed to analysis.

For **fluorescence microscopy**: Resuspend cell pellet without adding further buffer and transfer cells directly onto a slide. Cover cells with a cover slip and proceed immediately to microscopic analysis.

3. Examples of immunofluorescent staining with Anti-H-2K^k antibodies

Mouse 1881 pre-B cells stably transfected with the H-2K^k gene were mixed 1:3 with untransfected mouse 1881 pre-B cells. The cells were stained with Anti-H-2K^k antibodies conjugated to FITC (A), PE (B), or APC (C) and analyzed by flow cytometry using the MACSQuant[®] Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. Reference

- Lemke, H. and Hämmerling, G. J. (1981) Topographic Arrangement of H-2 Determinants Defined by Monoclonal Hybridoma Antibodies; in Hämmerling, G., Hämmerling, U., and Kearney, J. F. (eds.): Monoclonal Antibodies and T Cell Hybridomas. Elsevier, North Holland, Amsterdam, pp. 102–109.

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.

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