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1. Description

Components	<p>1 mL monoclonal Anti-H-2K^b/SIINFEKL antibodies, mouse conjugated to various dyes.</p> <p>PE 130-096-800</p> <p>Biotin 130-096-801</p> <p>or</p> <p>0.5 mL monoclonal Anti-H-2K^b/SIINFEKL antibodies, mouse</p> <p>pure – functional grade 130-096-810</p>
Clone	25-D1.16 (isotype: mouse IgG1).
Capacity	100 tests or up to 10 ⁹ total cells.
Product format	<p>The functional grade antibody is supplied at a concentration of 1 mg/mL.</p> <p>Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.</p> <p>Functional grade antibodies are supplied in phosphate-buffered saline (PBS), pH 7.2. Endotoxin levels have been tested and do not exceed 0.01 ng/μg of protein.</p> <p><i>The functional grade product contains no preservative and is sterile filtered; always handle under aseptic conditions.</i></p>
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 clone 25-D1.16 recognizes the ovalbumin-derived peptide SIINFEKL bound to the MHC class I allele H-2K^b.¹ The Anti-H-2K^b/SIINFEKL antibody is used to investigate antigen processing pathways *in vivo* and *in vitro*.

1.2 Applications

- Identification and enumeration of H-2K^b/SIINFEKL⁺ cells by flow cytometry or fluorescence microscopy.
- The Anti-H-2K^b/SIINFEKL pure – functional grade antibody is suited for functional assays, for example, blocking ovalbumin-specific CD8⁺ T cell responses.

1.3 Recommended antibody dilution

The recommended antibody dilution for all Anti-H-2K^b/SIINFEKL conjugates is **1:11 for up to 10⁷ cells/100 μL** of buffer for labeling of cells and analysis by flow cytometry.

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 mouse serum albumin, mouse serum, or fetal bovine serum (FBS). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Anti-Biotin antibodies conjugated to, e.g., APC (# 130-090-856) as secondary antibody reagent in combination with Anti-H-2K^b/SIINFEKL-Biotin.
- (Optional) CD11c-FITC (# 130-091-842), CD45-VioBlue[®] (# 130-092-910), or CD45R (B220)-FITC (# 130-091-829). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Mouse IgG1 isotype control antibodies conjugated to, e.g., FITC (# 130-092-213). For more information about isotype control antibodies refer to www.miltenyibiotec.com.
- (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.

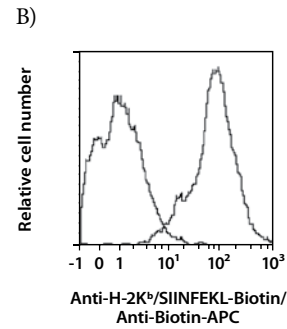
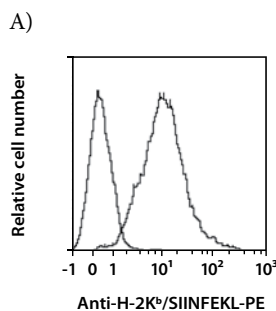
2. General protocol for immunofluorescent staining

▲ Volumes given below are for up to 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 2×10^7 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 μ L of buffer.
4. Add 10 μ L of the Anti-H-2K^b/SIINFEKL 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 $300 \times g$ for 10 minutes. Aspirate supernatant completely.
7. (Optional) If Anti-H-2K^b/SIINFEKL-Biotin was used, resuspend the cell pellet in 100 μ L of buffer, add 10 μ L of anti-biotin antibody, 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-H-2K^b/SIINFEKL antibodies

Spleen cells of a CD57BL/6 mouse were pulsed with or without the ovalbumin peptide_{257–264} SIINFEKL for two hours. Then cells were harvested, washed, and stained with Anti-H-2K^b/SIINFEKL conjugated to PE (A), CD11c-FITC, and CD45-VioBlue and analyzed by flow cytometry using the MACSQuant[®] Analyzer. Cells labeled with Anti-H-2K^b/SIINFEKL-Biotin (B) were stained with Anti-Biotin-APC (# 130-090-856) as well as CD11c-FITC and CD45-VioBlue. Gates were set on CD45⁺/CD11c⁺ cells. Histograms show pulsed positive cells (right peak), and as negative control unpulsed stained cells (left peak). Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. Reference

1. Porgador, A. *et al.* (1997) Localization, quantitation, and *in situ* detection of specific peptide-MHC class I complexes using a monoclonal antibody. *Immunity* 6: 715–726.

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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