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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.

1. Description

This product is for research use only.

Components	1 mL monoclonal Anti-HLA-E antibodies, human conjugated to:						
	<table border="0"> <tr> <td>PE</td> <td>130-096-849</td> </tr> <tr> <td>APC</td> <td>130-096-846</td> </tr> <tr> <td>Biotin</td> <td>130-096-890</td> </tr> </table>	PE	130-096-849	APC	130-096-846	Biotin	130-096-890
PE	130-096-849						
APC	130-096-846						
Biotin	130-096-890						
Clone	3D12 (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

- Antigen: HLA-E
- Expression patterns: The 3D12 monoclonal antibody recognizes the human HLA-E protein, a member of the human leukocyte antigen family. HLA-E is a non-classical MHC molecule (MHC-Ib) with limited polymorphisms, which is expressed on the cell surface in a heterodimeric complex with β 2-microglobulin. HLA-E is ubiquitously expressed and has a specialized role in NK cell recognition. It binds a restricted set of peptides, e.g., derived from other class I leader sequences. The complex is recognized by NKG2A on NK cells. This recognition protects the HLA-E-expressing cell from NK cell killing. Recently HLA-E recognition by a specialised subset of

CD8 T cells has been implicated in pathogen recognition as well as autoimmunity.^{1,2,3}

1.2 Applications

- Identification and enumeration of HLA-E⁺ cells by flow cytometry.

1.3 Recommended antibody dilution

The recommended antibody dilution for all Anti-HLA-E conjugates is **1:11 for up to 10⁷ cells/100 μ L** of buffer for labeling of cells and subsequent 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). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) Conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with Anti-HLA-E-Biotin.
- (Optional) For antibodies for additional staining or for isotype control, refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) for flow cytometric exclusion of dead cells without fixation.

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).

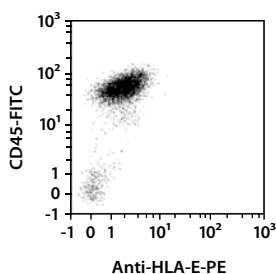
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10⁷ nucleated cells per 100 μ L of buffer.
4. Add 10 μ L of the Anti-HLA-E antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

▲ **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. (Optional) If Anti-HLA-E-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL 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. Example of immunofluorescent staining with Anti-HLA-E antibodies

PBMC were stained with Anti-HLA-E antibodies conjugated to PE as well as with CD45-FITC (# 130-080-202) 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.



For more examples please refer to the respective product page at www.miltenyibiotec.com/antibodies.

4. References

1. Pietra, G. *et al.* (2010) The Emerging Role of HLA-E-Restricted CD8⁺ T Lymphocytes in the Adaptive Immune Response to Pathogens and Tumors. *J. Biomed. Biotechnol.*: 907092.
2. Lu, L. *et al.* (2008) Generation and regulation of CD8⁺ regulatory T cells. *Cell Mol. Immunol.* 5 (6): 401–406.
3. Jiang, H. *et al.* (2010) HLA-E-restricted regulatory CD8⁺ T cells are involved in development and control of human autoimmune type 1 diabetes. *J. Clin. Invest.* 120 (10): 3641–3650.

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

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