

Contents

1. Description
 - 1.1 Background information
 - 1.2 Applications
 - 1.3 Recommended antibody dilution
 - 1.4 Reagent requirements
2. General protocol for immunofluorescent staining
3. Example of immunofluorescent staining with CD178 antibodies
4. References

1. Description

Components	1 mL monoclonal CD178 antibodies, human conjugated to:	
	PE	130-096-456
	APC	130-096-458
	Biotin	130-096-454
Clone	NOK-1 (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

Clone NOK-1 reacts with CD178, a type II transmembrane glycoprotein also known as Fas ligand or CD95L. It is expressed on activated lymphocytes (e.g., T cells, NK cells, monocytes, or granulocytes) as well as in immune-privileged sites (e.g., cornea or testis). CD178 can trigger apoptosis through trimerization of the Fas Receptor CD95. Thereby, CD178 and CD95 play a key role in the regulation of the immune system. Cleavage of membrane-bound CD178 from the cell surface via matrix metalloproteinases (such as MMP-7) generates soluble CD178 molecules.¹

1.2 Applications

- Identification and enumeration of CD178⁺ cells by flow cytometry.

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD178 conjugates is **1:11 for up to 10⁷ cells/100 µL** 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). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) Anti-Biotin antibodies conjugated to, e.g., PE (# 130-090-756) as secondary antibody reagent in combination with CD178-Biotin.
- (Optional) Mouse IgG1 isotype control antibodies conjugated to, e.g., PE (# 130-092-212). For more information about isotype control antibodies refer to www.miltenyibiotec.com.
- (Optional) Propidium Iodide Solution (# 130-093-233) for flow cytometric exclusion of dead cells without fixation.

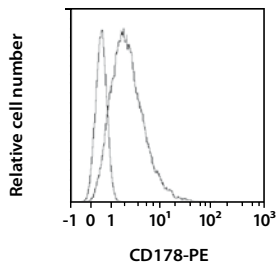
2. General protocol for immunofluorescent staining

▲ Volumes given below are for up to 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 μL of buffer.
4. Add 10 μL of the CD178 antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator ($2-8^\circ\text{C}$).
▲ **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 CD178-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 CD178 antibodies

Human L5178 cells were stained with CD178 antibodies conjugated to PE 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.

4. References

1. Kayagaki, N. *et al.* (1995) Metalloproteinase-mediated release of human Fas ligand. *J. Exp. Med.* 182: 1777–1783.
2. Suda, T. *et al.* (1993) Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family. *Cell* 75: 1169–1178.
3. Janssen, O. *et al.* (2003) CD95 ligand – death factor and costimulatory molecule? *Cell Death Differ.* 10: 1215–1225.

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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