Miltenyi Biotec

Anti-Ki-67 antibodies
human and mouse

Contents

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 Monoclonal Anti-Ki-67 antibodies, human and mouse:

Conjugate	Order no. 1 mL (100 tests)	Order no. 300 µL (30 tests)
FITC	130-100-339	130-100-340
PE	130-100-289	130-100-290
APC	130-100-330	130-100-332
PE-Vio® 615	130-107-150	130-107-203
PE-Vio® 770	130-100-288	130-100-291
PerCP-Vio® 700	130-100-292	130-100-293

Capacity 1 mL: 100 tests or up to 10^9 total cells

300 µL: 30 tests or up to 3×10^8 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: Ki-67
- Expression patterns: Clone REA183 recognizes Ki-67 antigen, a nuclear and nucleolar protein, which is strictly associated with cell proliferation. Two Ki-67 isoforms with molecular weight, 395 and 345 kDa are known. Both isoforms contain 16 "Ki-67 repeat" sequences, each of which includes a conserved 66 bp "Ki-67 motif". Expression of Ki-67 protein is present during all active phases of the cell cycle (G(1), S, G(2), and mitosis), but is absent from resting cells (G(0)). Due to strict association of Ki-67 with proliferating cells, Ki-67 is often used as an indicator of the "growth fraction" of a given cell population. The N-terminal portion of Ki-67 contains a forkhead associated (FHA)1 domain, which is involved in interaction with proteins such as Hklp2 and NIFK. Additional information: Clone REA183 displays negligible binding to Fc receptors.

1.2 Applications

- Identification and enumeration of Ki-67$^+$ cells by flow cytometry.

1.3 Recommended antibody dilution

The recommended antibody dilution for all Anti-Ki-67 antibodies is **1:11 for up to 10^7 cells/100 µL** of buffer for labeling of cells and subsequent analysis by flow cytometry.

1.4 Reagent requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, and 1% bovine serum albumin (BSA). Keep buffer cold (2–8 °C).
- 70% ethanol
- (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.
- (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×g for 10 minutes. Aspirate supernatant completely.
3. Loosen the cell pellet by vortexing.
4. Add 5 mL of cold 70% ethanol dropwise while vortexing.

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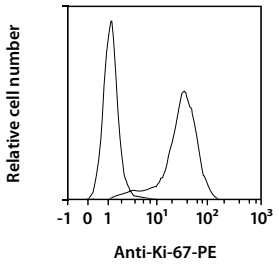
5. Mix well and incubate for 1 hour at –20 °C.
6. Wash cells by adding 40 mL of buffer twice and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
8. Add 10 µL of the Anti-Ki-67 antibody.
9. Mix well and incubate for 20 minutes in the dark at room temperature (19–25 °C).
10. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
11. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Example of immunofluorescent staining with Anti-Ki-67 antibodies

Jurkat cells were fixed, permeabilized, and stained with Anti-Ki-67 antibodies conjugated to PE and analyzed by flow cytometry using the MACSQuant® Analyzer. The specificity of the conjugated antibodies was confirmed by blocking the binding to the ligand, using pure unconjugated antibodies (left peak). Cell debris and dead cells were excluded from the analysis based on scatter signals.



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

4. References

1. Verheijen, R. *et al.* (1989) Ki-67 detects a nuclear matrix-associated proliferation-related antigen. II. Localization in mitotic cells and association with chromosomes. J. Cell Sci. 92: 531–540.
2. Takagi, M. *et al.* (2001) A novel nucleolar protein, NIFK, interacts with the forkhead associated domain of Ki-67 antigen in mitosis. J. Biol. Chem. 276: 25386–25391.

Refer to www.miltenyibiotec.com for all data sheets and protocols.

Warranty

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