Monoclonal Mouse Anti-Human Ki-67 Antigen/FITC

Clone MIB-1

Code No. F 7268

For research use only. Not for use in diagnostic procedures.

Recommended use

Monoclonal Mouse Anti-Human Ki-67 Antigen/FITC, is recommended for use in flow cytometry for identification of cells expressing the Ki-67 antigen.

Introduction

The Ki-67 antigen is a nuclear protein defined by its reactivity with the monoclonal antibody from the Ki-67 clone (1, 2). During interphase, the Ki-67 antigen can be exclusively detected within the nucleus, whereas in mitosis most of the protein is relocated to the surface of the chromosomes (1). Two isoforms of 345 and 395 kDa have been identified (3). The complete gene locus of the Ki-67 antigen has been sequenced. The size of the gene is approximately 30 000 base pairs organized in 15 exons with sizes from 67 to 6845 base pairs, and in 14 introns with sizes from 87 to 3569 base pairs. The gene is located on chromosome 10 (4).

The Ki-67 antigen is expressed in all proliferating cells during late G_1, S, M and G_2 phases of the cell cycle while cells in the G_0 (non-cycling) phase consistently lack the Ki-67 antigen. Un-stimulated normal human cells do not express the Ki-67 antigen (1, 2).

Flow cytometry has been demonstrated to be a useful method for detecting Ki-67 antigen and assessing cellular proliferation in tumour cells as an alternative to S-phase cell cycle determination, continuous ^{3}H-thymidine labelling, acridine orange and bromodeoxyuridine staining (5, 6).

Reagent provided

Purified monoclonal mouse antibody conjugated with fluorescein isothiocyanate isomer 1 (FITC). The conjugate is provided in liquid form in buffer containing 1% bovine serum albumin (BSA) and 15 mmol/L NaN_3, pH 7.2. Each vial contains 100 tests (10 µL of conjugate for up to 10^6 cells).

Clone: MIB-1. Isotype: IgG1, kappa. Conjugate concentration mg/L: See label on vial.

Immunogen

Human recombinant peptide corresponding to a 1002 bp Ki-67 cDNA fragment (7).

Specificity

Anti-Ki-67 Antigen, MIB-1, reacts with a formalin-resistant epitope and can thus be used on cells obtained from formalin-fixed, paraffin-embedded tissues. In Western blotting of lysates of the multiple myeloma cell line, IM-9, Anti-Ki-67 Antigen, MIB-1, labels bands of 345 and 395 kDa, identical to the bands labelled by the original Ki-67 antibody (7). Flow cytometric analysis has shown that Anti-Ki-67 Antigen, MIB-1, reacts negatively or weakly with un-stimulated cells from normal healthy donors (6). The antibody labels proliferating cancer cell lines, e.g. HeLa, RAJI and IM-9 cells. Anti-Ki-67 Antigen, MIB-1, labels also non-cancer cell lines, e.g. the human urothelial cell line HCV29 in growth phase (6), and a subpopulation of CD4+CD45R0+ T lymphocytes from HIV-infected patients (8).

Precautions

1. The device is not intended for clinical use including diagnosis, prognosis, and monitoring of a disease state, and it must not be used in conjunction with patient records or treatment.

2. This product contains sodium azide (NaN_3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.

3. As with any product derived from biological sources, proper handling procedures should be used.

Storage

Store in the dark at 2-8 °C. Do not use after expiration date stamped on vial. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the product is suspected, contact our Technical Services.

Staining procedure

1. Harvest cells and determine total number present. Wash twice in 0.01 mol/L PBS.
2. If required, perform staining of cell surface antigens using appropriate directly conjugated monoclonal antibodies at this stage. Following staining, wash cells once in 0.01 mol/L PBS and discard supernatant. When performing multi-parameter flow cytometric analysis employing DNA-staining dyes, it is recommended to use propidium iodide in combination with APC-conjugated antibodies, and DRAQ5 in combination with RPE-conjugated antibodies.
3. Add Dako IntraStain Reagent A, Fixative, code No. K 2311, using 50 µL per 1 x 10^6 cells. Mix gently by using a vortex mixer to ensure that the cells are in suspension.
4. Incubate at room temperature for 10 minutes.

5. Add 2 mL 0.01 mol/L PBS and mix gently by using a vortex mixer.
6. Centrifuge at 300 x g for 5 minutes, then aspirate the supernatant, leaving approximately 50 µL of fluid.
7. Mix thoroughly by using a vortex mixer to ensure that the cells are in suspension and add Dako IntraStain Reagent B, Permeabilization, plus Nonidet P40 (1 µL to 1 mL of IntraStain Reagent B, code No. K 2311) using 50 µL per 1 x 10^6 cells.
8. Add 10 µL of F 7268. Mix gently by using a vortex mixer to ensure that the cells are in suspension.
9. Use a non-reactive monoclonal antibody of the same isotype, and conjugated with the same fluorochrome, e.g. Dako code No. X 0927, as a negative control.
10. Incubate in the dark at 4 °C for 15 minutes.
11. Repeat steps 5 and 6.
12. Wash once in 0.01 mol/L PBS, and resuspend in 0.3 mL 1.0% paraformaldehyde (fixative) in 0.01 mol/L PBS, pH 7.4.
13. Analyse on a flow cytometer.

Please note that a region defined by code No. X 0927, Negative Control, in a negative cell population does not always include all MIB-1 negative cells, therefore the use of several types of negative control cells is strongly recommended.

References

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4. Duchrow M, Schlüter C, Wohlenberg C, Flad H-D, Gerdes J. Molecular characterization of the gene locus of the human cell proliferation-associated nuclear protein defined by monoclonal antibody Ki-67. Cell Prolif 1996;29:1-12.
5. Keng PC, Siemann DW. Measurement of proliferation activities in human tumor models: a comparison of flow cytometric methods. Radiat Oncol Invest 1998;6:120-7.
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7. Key G, Becker MHG, Baron B, Duchrow M, Schlüter C, Flad H-D, et al. New Ki-67-equivalent murine monoclonal antibodies (MIB 1-3) generated against bacterially expressed parts of the Ki-67 cDNA containing three 62 base pair repetitive elements encoding for the Ki-67 epitope. Lab Invest 1993;68:629-36.
8. Combadière B, Blanc C, Taisheng L, Carcelain G, Delaugerre C, Calvez V, et al. $CD4^+Ki67^+$ lymphocytes in HIV-infected patients are effector T cells accumulated in the G1 phase of the cell cycle. Eur J Immunol 2000;30:3598-3603.

Explanation of symbols

REF	Catalogue number		Keep away from sunlight (consult storage section)		Manufacturer
	Consult instructions for use	LOT	Batch code	EC REP	Authorized representative in the European Community
2 °C 8 °C	Temperature limitation		Use by		

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Revision 2020.11