Monoclonal Mouse
Anti-Human Topoisomerase IIα
Clone Ki-S1
Code No. M7186

Recommended use

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

Monoclonal Mouse Anti-Human Topoisomerase IIα, Clone Ki-S1, is recommended for use in immunocytochemistry for identification of cells expressing Topoisomerase IIα.

Introduction

DNA topoisomerases are ubiquitous enzymes that control DNA topology by cleaving and rejoining DNA strands. There are two major types of DNA topoisomerases, topo I and II, which are functionally distinct. Topo I cuts one strand of DNA, whereas topo II cuts both strands of DNA at one time in an ATP-dependent reaction. Studies on topo II from vertebrates, including humans, have revealed the existence of two isoforms. Topo IIα with a molecular mass of 170 kDa, and topo IIβ with a molecular mass of 180 kDa. The two isoforms normally exist as homodimers, but a small proportion of αβ heterodimers has been detected in cultured cells. The two isoforms are highly similar, but genetically distinct, and display different expression patterns (1). Topo IIα is expressed in proliferating cells in late S phase, and with a peak in G_2-M phases , where it is believed to be the primary mediator of chromosome condensation. Topo IIα is not expressed in the G_0 phase, representing resting and non-proliferating cells. In contrast, topo IIβ is constantly expressed once the cells enter the cell cycle and is still present in the G_0 phase (2).

Reagent provided

Monoclonal mouse antibody provided in liquid form as purified IgG from ascitic fluid, in 0.05 mol/L Tris/HCl, 15 mmol/L NaN_3, 1% bovine serum albumin, pH 7.2.

Clone: Ki-S1 (3). Isotype: IgG2a, kappa.

Mouse IgG concentration: See label on vial.

Immunogen

Lysate of the human histiocytic lymphoma cell line U-937 (3).

Specificity

In Western blotting of extract of yeast strain BJ 201 transfected with plasmid pHT 300α containing the human topoisomerase IIα gene, the antibody labels a band of 170 kDa corresponding to human topoisomerase IIα. No labelling was observed with extracts of yeast strain BJ 201 expressing *S. pombe* topoisomerase II (4).

In Western blotting of human HL-60 cell lysates containing equal amounts of topo IIα and topo IIβ, the antibody only labels a 170 kDa band corresponding to the α isoform. The epitope recognized was found to be within the last 495, and most likely within the last 29, carboxyl-terminal amino acid residues of the protein (4).

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 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 antibody is suspected, contact our Technical Services.

Specimen preparation

Paraffin sections: The antibody can be used for labelling paraffin-embedded tissue sections fixed in formalin, whereas methanol/acetic acid fixation destroys the antigen (3). Pre-treatment of tissues with heat-induced epitope retrieval is required. For tissues fixed in formalin, optimal results are obtained with Dako Target Retrieval Solution, code No. S1700, Dako Target Retrieval Solution, High pH, code No. S3308, Dako Target Retrieval Solution. Citrate, pH 6.0, code No. S2369, or Dako Target Retrieval Solution, pH 9.0, code No. S2368. Pre-treatment of tissues with proteinase K was found inefficient. The tissue sections should not dry out during the treatment or during the following immunocytochemical staining procedure.

Frozen sections and cell preparations: The antibody can be used for labelling acetone-fixed, frozen sections (3).

Staining procedure

Dilution: Monoclonal Mouse Anti-Human Topoisomerase IIα, code No. M7186, may be used at a dilution range of 1:50-1:100 when applied on formalin-fixed, paraffin-embedded sections of human tonsil and using 20 minutes heat-induced epitope retrieval in Dako Target Retrieval Solution, code No. S1700, and 30 minutes incubation at room temperature with the primary antibody. Optimal conditions may vary depending on specimen and preparation method, and should be determined by each individual laboratory. The recommended negative control is Dako Mouse IgG2a, code No. X0943, diluted to the same mouse IgG concentration as the primary antibody.

Visualization: DAKO LSAB™+/HRP kit, code No. K0679, and DAKO EnVision™+/HRP kits, code Nos. K4004 and K4006, are recommended. For frozen sections and cell preparations, the Dako APAAP kit, code No.

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K0670, is a good alternative if endogenous peroxidase staining is a concern. Follow the procedure enclosed with the selected visualization kit.

Product-specific limitations

A single major cytoplasmic co-reactivity not associated with proliferation was observed in the Langerhans islands of the pancreas, and a weak nuclear background staining not associated with proliferation was occasionally seen in chrondocytes of hyaline cartilage (1).

Performance characteristics

Cells labelled by the antibody display a nuclear staining pattern, and only occasionally is a weak cytoplasmic staining observed (1).

References

1. Bakshi RP, Galande S, Muniyappa K. Functional and regulatory characteristics of eukaryotic type II DNA topoisomerase (review). Crit Rev Biochem Mol Biol 2001;36:1-37.
2. Woessner RD, Mattern MR, Mirabelli CK, Johnson RK, Drake FH. Proliferation- and cell cycle-dependent differences in expression of the 170 kilodalton and 180 kilodalton forms of topoisomerase II in NIH-3T3 cells. Cell Growth Differ 1991;2:209-14.
3. Kreipe H, Heidebrecht HJ, Hansen S, Röhlk W, Kubbies M, Wacker HH, et al. A new proliferation-associated nuclear antigen detectable in paraffin-embedded tissues by the monoclonal antibody Ki-S1. Am J Pathol 1993;142:3-9.
4. Boege F, Andersen A, Jensen S, Zeidler R, Kreipe H. Proliferation-associated nuclear antigen Ki-S1 is identical with topoisomerase IIα. Am J Pathol 1995;146:1302-8.

Explanation of symbols

REF	Catalogue number	2 °C 8 °C	Temperature limitation		Use by
	Consult instructions for use	LOT	Batch code		Manufacturer

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