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Not for use in diagnostic procedures.

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MONOCLONAL ANTIBODY

Anti-Phospho-DNA Topoisomerase IIα (Thr1342) (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M025-3	3D4	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: Topoisomerase II (Topo II) is a nuclear enzyme that regulates the topological states of DNA by transient breakage and rejoining double-stranded DNA, catalyzing the decatenation and unknotting of topologically linked DNA circles and the relaxation of supercoiled DNA. In mammalian cells, Topo II consists of two isozymes, Topo IIα (170 kDa) and Topo IIβ (180 kDa). Expression and localization of each isoform are distinct and stage specific during the cell cycle. Topo IIβ is expressed constantly throughout cell cycle, whereas the expression of Topo IIα is cell cycle-regulated, peaking in G_2 to M phase and declining to a minimal level at the end of M phase. It is considered that Topo IIα plays an essential role in cell proliferation, especially during late S to M phase. Threonine 1342 in human Topo IIα is phosphorylated throughout the cell cycle. Phosphorylation level of threonine 1342 in G_2 to M phase is as twice as much in G_1 or S phase. Anti-phospho-DNA Topoisomerase IIα antibody recognize phosphorylated threonine residue, PT 1342 in human Topo IIα specifically and does not react with non-phosphorylated form.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma (clone 3D4) was established by fusion of mouse myeloma cell SP2/0-Ag14 with Balb/c mouse splenocyte immunized with the human Topo IIα synthetic phosphopeptide corresponding to FSDFDEK(p)TDDEDFVPC (1335-1349 aa)

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with human phospho-Topo IIα (170 kDa) on Western blotting.

INTENDED USE:
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APPLICATIONS:
Western blotting; 1-10 µg/mL
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; 10 µg/mL
Flow cytometry; Not tested

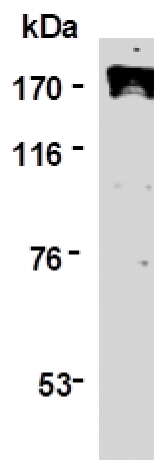
Detailed procedure is provided in the following **PROTOCOLS**.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Jurkat	WR19L	PC12
Reactivity on WB	+	-	-

REFERENCES:
1) Sato, T., *et al., Cancer Res*. **65**, 6950-6956 (2005) [WB]
2) Agostinho, M., *et al., Mol. Biol. Cell* **15**, 2388-2400 (2004)
3) Ishida R., *et al., J. Biol. Chem*. **271**, 30077-30082 (1996)

Clone 3D4 is used in these references.



Western blotting analysis of phospho-DNA Topoisomerase IIα (Thr1342) expression in Jurkat cells using M025-3.

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOLS:

SDS-PAGE & Western blotting

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer [50 mM Tris-HCl (pH 7.2), 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 3 minutes and centrifuge. Load 10 µL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). See the manufacturer's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
7) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
9) Incubate the membrane with 1:10,000 of Anti-IgG (H+L chain) (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (10 minutes x 3).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 3 minutes.
14) Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; Jurkat)

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (For example, spread 1 x 10^4 cells for one slide, then incubate in a CO_2 incubator overnight.)
2) Fix the cells by immersing the slide in Acetone for 10 minutes at room temperature.
3) The glass slide was washed with PBS 3 times.
4) Immerse the slide in PBS containing 0.2% Triton X-100 for 10 minutes at 4°C.
5) The glass slide was washed with PBS 3 times.
6) Cover the cells with blocking buffer (0.2% BSA in PBS) for 10 minutes to minimize non-specific adsorption of the antibodies to the cover slip.
7) Remove the blocking buffer.
8) Add primary antibody diluted with as suggested in the **APPLICATIONS** onto the cells and incubate for 1 hour at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
9) The glass slide was washed with PBS 3 times.
10) Add 100 µL of Anti-IgG (Mouse) pAb-FITC diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
11) The glass slide was washed with PBS 3 times.
12) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
13) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; HEp-2)

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