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MONOCLONAL ANTIBODY

Anti-DNA Topoisomerase IIα (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M042-3	1C5	Mouse IgG2a	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.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell Sp2/0-Ag14 with Balb/c mouse splenocyte immunized with the recombinant human Topoisomerase IIα protein corresponding to C-terminal 182 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 detects 170 kDa of human Topo IIα on Western blotting with total cell lysate from human cell lines.

APPLICATIONS:
Western blotting; 0.1-0.5 µg/mL
Immunoprecipitation; 1-10 µg/200-300 µL of cell extract
Immunohistochemistry; 1 µg/mL
*Suitable for use in paraffin sections.
Heat treatment is necessary for paraffin embedded sections.
Microwave oven; twice for 10 minutes each in 10 mM citrate buffer (pH 6.5).
Immunocytochemistry; 1-10 µg/mL
Flow cytometry; 0.1-1 µg/mL (final concentration)

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells	Jurkat, Raji, K562, A431, HEp-2	WR19L, NIH/3T3	PC12 Rat-1	BHK
Reactivity on WB	+	-	-	-

INTENDED USE:
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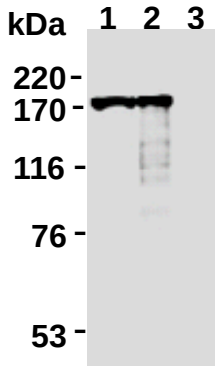
REFERENCES:
1) Nakagawa, T., *et al.*, *Cancer Res*. **64**, 4826-4832 (2004)
2) Mensah-Osman, E.J., *et al.*, *Mol. Cell Ther.* **14**, 1321-1326 (2002)
3) Escargueil, A.E., *et al.*, *J. Biol. Chem.* **275**, 34710-34718 (2000)
4) Meyer, K. N., *et al.*, *J. Cell Biol.* **136**, 775-788 (1997)
5) Sasano, K., *et al.*, *Cell Technology (in japanese)* **16**, 1613-1617 (1997)
6) Goswami, P.C., *et al.*, *Mol. Cell. Biol*. **16**, 1500-1508 (1996)
7) Woessner, R.D., *et al.*, *Cell Growth Differ.* **2**, 209-214 (1991)

Clone 1C5 is used in the reference number 1).

PROTOCOLS:
SDS-PAGE & Western blotting
1) Wash the cells 3 times with PBS and suspend with 10 volumes 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] (10 minutes x 3).
9) Incubate the membrane with the 1:10,000 Anti-IgG (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 controls for Western blotting; Jurkat, Raji)



Western blotting analysis of DNA Topoisomerase IIα expression in Jurkat (1), Raji (2) and WR19L (3) using M042-3.

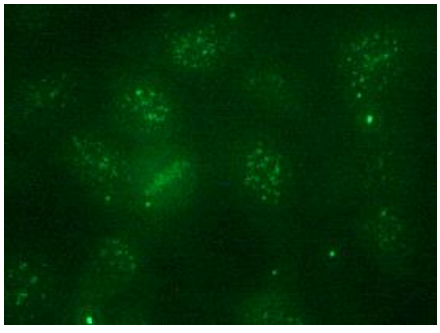
Immunoprecipitation

1) Wash the cells 3 times with PBS and suspend with 10 volumes 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.
3) Add primary antibody as suggested in the **APPLICATIONS** into 300 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 µL of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
4) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant using a pipettor without disturbing the beads.
5) Resuspend the beads with cold Lysis buffer.
6) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant.
7) Repeat steps 5)-6) 3-5 times
8) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (For example, spread 10^4 of cells per one well, then incubate in a CO_2 incubator overnight.)
2) Fix the cells by immersing the slide in Acetone for 10 minutes on ice.
3) Air dry the slides.
4) Add the primary antibody diluted with PBS 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.)
5) Prepare a wash container such as a 500 mL beaker with a magnetic stirrer. Then wash the cultured cells on the glass slide by soaking the slide with a plenty of PBS in the wash container for 5 minutes. Take care not to touch the cells. Repeat another wash once more.
6) Add 30 µL of 1:100 Anti-IgG (Mouse) pAb-FITC (Beckman Coulter, code no. IM0819) diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
7) Wash the slide in a plenty of PBS as in the step 5).
8) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
9) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; HEp-II)

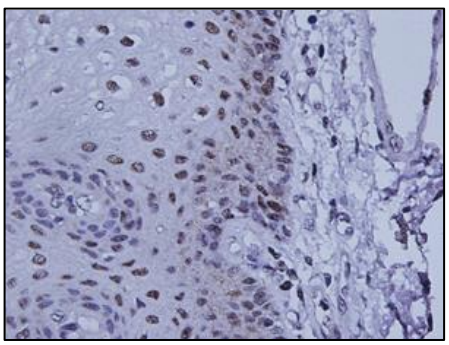


Immunocytochemical detection of DNA Topoisomerase IIα on acetone fixed HEp-II with M042-3.

Immunohistochemical staining for paraffin-embedded sections

1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Heat treatment
   Heat treatment by Microwave:
   Place the slides put on staining basket in 500 mL beaker with 500 mL of 10 mM citrate buffer (pH 6.5). Cover the beaker with plastic wrap, then process the slides twice for 10 minutes each at 500 W with microwave oven. Let the slides cool down in the beaker at room temperature for about 40 minutes.
5) Remove the slides from the citrate buffer and cover each section with 3% H_2O_2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
6) Remove the slides from PBS, wipe gently around each section and cover tissues with blocking buffer (20 mM HEPES, 1% BSA, 135 mM NaCl) for 5 minutes to block non-specific staining. Do not wash.
7) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with blocking buffer as suggested in the **APPLICATIONS**.
8) Incubate the sections for 1 hour at room temperature.
9) Wash the slides 3 times in PBS for 5 minutes each.
10) Wipe gently around each section and cover tissues with ENVISION+Dual Link (Agilent, code no. K4063). Incubate for 1 hour at room temperature. Wash as in step 9).
11) Visualize by reacting for 10 minutes with DAB substrate solution. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
12) Wash the slides in water for 5 minutes.
13) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
14) Now ready for mounting.

(Positive controls for Immunohistochemistry; human tonsil, stomach, lung)



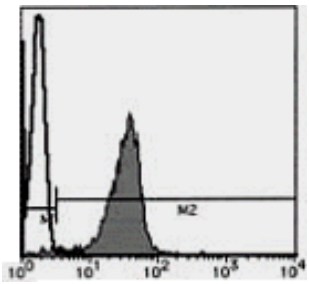
Immunohistochemical detection of DNA Topoisomerase IIα on paraffin embedded section of human stomach with M042-3.

Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all steps described below.

1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN_3].
   *Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.
2) Add 200 µL of 70% ethanol to the cell pellet after tapping. Mix well, then permeabilize the cells for 30 minutes at -20°C.
3) Wash the cells 3 times with washing buffer.
4) Add 10 µL Clear Back (human Fc receptor blocking reagent, MBL, code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature (20~25°C).
5) Add 30 µL of the primary antibody at the concentration of as suggested in **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 30 minutes at room temperature.
6) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
7) Add 30 µL of 1:40 Anti-IgG (Mouse) pAb-FITC (Beckman Coulter, code no. IM0819) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
8) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
9) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; Jurkat)



Flow cytometric analysis of DNA Topoisomerase IIα expression in Jurkat. *Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of M042-3 to the cells.*

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