Protocol

DNA-topoisomerase IIα, mAb (1C5)

Catalog #: ADI-KAM-CC210

Code No.	Clone	Subclass	Quantity	Concentration
M042-3	1C5	Mouse IgG2a	100µl	1 mg/ml

USE FOR RESEARCH PURPOSES ONLY

Unless otherwise specified expressly on the packaging, all products sold hereunder are intended for and may be used for research purposes only and may not be used for food, drug, cosmetic or household use or for the diagnosis or treatment of human beings. Purchase does not include any right or license to use, develop or otherwise exploit these products commercially. Any commercial use, development or exploitation of these products or development using these products without the express written authorization of Enzo Life Sciences, Inc. is strictly prohibited. Buyer assumes all risk and liability for the use and/or results obtained by the use of the products covered by this invoice whether used singularly or in combination with other products.

LIMITED WARRANTY; DISCLAIMER OF WARRANTIES

These products are offered under a limited warranty. The products are guaranteed to meet all appropriate specifications described in the package insert at the time of shipment. Enzo Life Sciences' sole obligation is to replace the product to the extent of the purchasing price. All claims must be made to Enzo Life Sciences, Inc., within five (5) days of receipt of order. THIS WARRANTY IS EXPRESSLY IN LIEU OF ANY OTHER WARRANTIES OR LIABILITIES, EXPRESS OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, AND NON-INFRINGEMENT OF THE PATENT OR OTHER INTELLECTUAL PROPERTY RIGHTS OF OTHERS, AND ALL SUCH WARRANTIES (AND ANY OTHER WARRANTIES IMPLIED BY LAW) ARE EXPRESSLY DISCLAIMED.

TRADEMARKS AND PATENTS

Several Enzo Life Sciences products and product applications are covered by US and foreign patents and patents pending. Enzo is a trademark of Enzo Life Sciences, Inc.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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 (170kDa) and Topo IIß (180kDa). Expression and localization of each isoform are distinct and stage specific during the cell cycle. Topo IIß is expressed constantly throughout cell cycle, whereas the expression of Topo IIα is cell cycle-regulated, peaking in G2 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

100µg IgG in 100µl 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 170kDa of human Topo IIα on Western blotting with total cell lysate from human cell lines.

APPLICATIONS

- Western blotting; 0.1-0.5µg/ml for chemiluminescence detection system
- Immunoprecipitation; 1-10µg/200-300µl of cell extract
- Immunohistochemistry; 1µg/ml

  *Suitable for use in paraffin sections.

  - Heat treatment is necessary for paraffin embedded sections.
  - Microwave oven; 2 times for 10 minutes each in 10mM citrate buffer (pH 6.5).
- Immunocytochemistry; 1-10µg/ml
- Flowcytometry; 0.1-1µg/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	PC12M Rat-1	BHK
Reactivity on WB	+	-	-	-

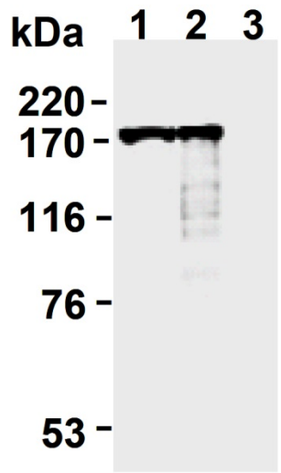
INTENDED USE

For Research Use Only. Not for use in diagnostic procedures.

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 volume of cold Lysis buffer (50mM Tris-HCl, pH 7.2, 250mM NaCl, 0.1% NP-40, 2mM 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 12,000xg 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'ssample buffer.
4. Boil the samples for 3minutes and centrifuge. Load 10µl 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 1mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25mM Tris, 190mM glycine, 20% MeOH). 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 PBS, pH 7.2 containing 1% skimmed milk 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 times).
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 times).
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)

IMMUNOPRECIPITATION

1. Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50mM Tris-HCl pH 7.2, 250mM NaCl, 0.1% NP-40, 2mM 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 300µl of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20µl of 50% protein Aagarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
4. Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5. Resuspend the beads in 20µl of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10µl/lane for the SDS-PAGE analysis.

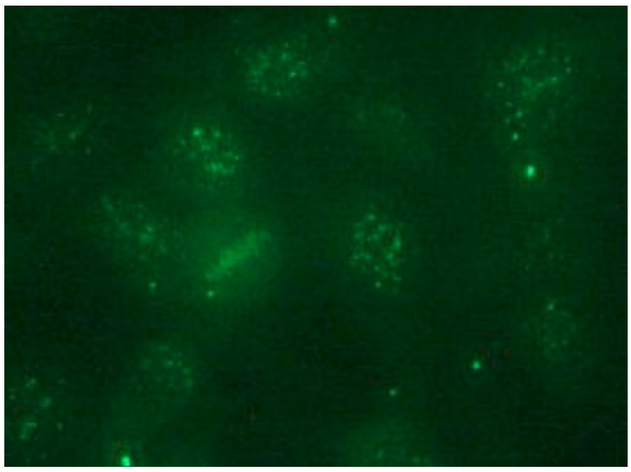
(See SDS-PAGE & Western blotting.)

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 for one night.)
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 500ml 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 30µl of 1:100 Anti-IgG(Mouse) pAb-FITC (MBL; code no. IM-0819) 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

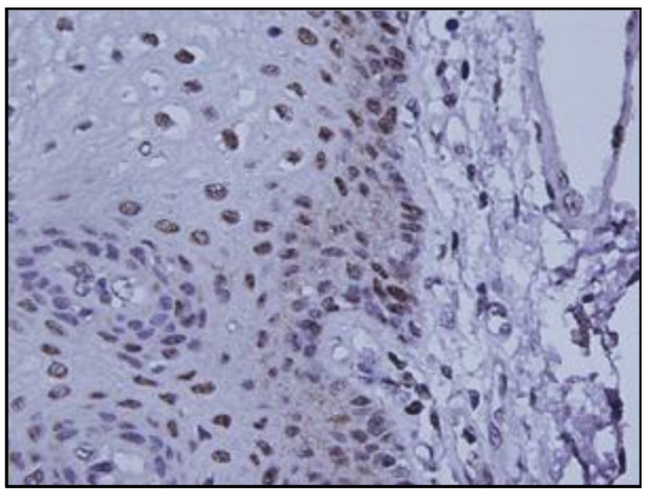
1. Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2. Wash the slides with Ethanol 3 times for 3-5 minutes each.
3. Wash the slides with PBS 3 times for 3-5 minutes each.
4. Heat treatment by Microwave:

   Place the slides put on staining basket in 500ml beaker with 500ml of 10mM citrate buffer (pH 6.2). Cover the beaker with plastic wrap, then process the slides 2 times 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 (20mM HEPES, 1% BSA, 135mM NaCl) for 5minutes 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 (DAKO; 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 (DAKO; code no. K3465). *DAB is asuspect 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 minute seach, 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)



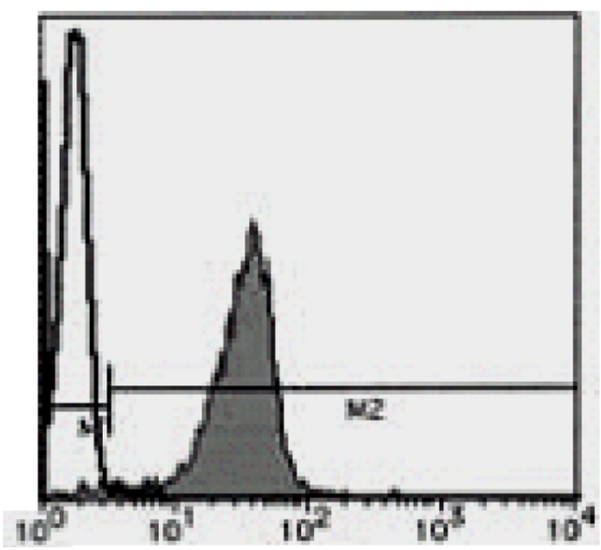
Immunohistochemical detection of DNA Topoisomerase IIα on paraffin embedded section of human stomach with M042-3.

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% NaN3].
2. Add 200µl 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 10µl of normal goat serum containing 1mg/ml normal human IgG and 0.1% NaN3to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature (20~25°C).
5. Add 30µl 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 1ml of the washing buffer followed by centrifugation at 500 x gfor 1 minute at room temperature. Remove supernatant by careful aspiration.
7. Add 30µlof 1:40 Anti-IgG(Mouse) pAb-FITC (MBL:code no. IM-0819) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
8. Add 1mlof the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
9. Resuspend thecells with 500µl 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.

NOTES

GLOBAL HEADQUARTERS
Enzo Life Sciences Inc.
10 Executive Boulevard
Farmingdale, NY 11735
Toll-Free:1.800.942.0430
Phone:631.694.7070
Fax: 631.694.7501
info-usa@enzolifesciences.com

EUROPE/ASIA
Enzo Life Sciences (ELS) AG
Industriestrasse 17
CH-4415 Lausen
Switzerland
Phone:+41/0 61 926 89 89
Fax:+41/0 61 926 89 79
info-ch@enzolifesciences.com

For local distributors and detailed product information visit us online:
www.enzolifesciences.com