Datasheet for ABIN5542012

anti-Topoisomerase II alpha antibody (pThr1342)

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Overview

Quantity:	0.1 mL
Target:	Topoisomerase II alpha (TOP2A)
Binding Specificity:	AA 1335-1349, pThr1342
Reactivity:	Human
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This Topoisomerase II alpha antibody is un-conjugated
Application:	Western Blotting (WB), Immunofluorescence (IF), Immunocytochemistry (ICC)

Product Details

Immunogen:	Human Topo IIalpha sythetic peptide
Sequence:	SDFDEK(p)TDDEDFVPC
Clone:	3D4
Isotype:	IgG1
Specificity:	This antibody reacts with the Topo IIα.
Cross-Reactivity (Details):	Does not work with Mouse (WR19L) and Rat (PC12).
Purification:	Protein A agarose

Target Details

Target:	Topoisomerase II alpha (TOP2A)

Target Details

Alternative Name:	top2a (TOP2A Products)
Background:	Topo IIalpha plays important roles in synthesis and transcription of DNA as well as chromosomal segregation during mitosis. It is reported to be a sensitive and specific marker of late S-, G2- and M-phases in transformed and developmentally regulated normal cells. Note that Ki67/M1B1 is also present in G1-phase. Topo IIalpha is also implicated in drug resistance of tumor cells.
Pathways:	Cell Division Cycle, Mitotic G1-G1/S Phases

Application Details

Application Notes:	Western blot: 1-10 µg/mL for chemiluminescence detection system.. Immunocytochemisty: 10 µg/mL.
Protocol:	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 appropria te protease inhibitors. Incubate it at 4 o 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 o 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, 2 0 % 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 o C. 7) Incubate the membrane with primary antibody d iluted with PBS, pH 7.2 containing 1 % skimmed milk as suggest ed 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 times). 9) I ncubate the membrane with the 1:10,000 HRP - conjugated anti - mouse IgG 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 control for Western

Application Details

	blotting Jurkat) Immunocytochemistry 1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 10 4 the cells for one slide, then incubate in a C O 2 incubator for one night.) 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 o C. 5) The glass slide was w ashed 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 suggest ed in the APPLICATIONS onto the cells and incubate for 1 hour at room temperature. (Optimization of antibody concentration or incubation condition are recommended if necessary.) 9) The glass slide was washed with PBS 3 times. 10) Add 100 µ L of 1:160 FITC-conjugated anti mouse IgG 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 HeLa)
Restrictions:	For Research Use only

Handling

Format:	Liquid
Buffer:	PBS containing 50 % glycerol, pH 7.2. No preservative is contained.
Preservative:	Azide free
Storage:	-20 °C
Storage Comment:	Store undiluted (in aliquots) at -20°C. Avoid repeated freezing and thawing. Shelf life: one year from despatch.
Expiry Date:	12 months