abcam

Product datasheet

Anti-Topoisomerase I antibody - ChIP Grade ab3825

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Overview

Product name	Anti-Topoisomerase I antibody - ChIP Grade
Description	Rabbit polyclonal to Topoisomerase I - ChIP Grade
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, IHC-Fr, WB, IP, ChIP
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Recombinant fragment, corresponding to amino acids 401 - 600 of Human Topoisomerase I.
Positive control	HeLa cells.
General notes	This product can be used as part of an assay for sumoylation activity. Human Aos 1 + Uba 2 (ab3804), Ubc 9 (ab3803) and Sumo 1 (ab3801) can be used to promote in vitro sumoylation of a sumoylation marker (human Topoisomerase I protein fragment) (ab3828). The reaction products can be detected using our Sumo 1 (ab3819 and ab3824) and Topoisomerase I (ab3825) antibodies. Sumoylation assays are carried out in a final volume of 20µl in reaction conditions (20 mM Hepes pH 7.5, 5mM MgCl2, 2mM ATP). This antibody is equivalent to the antibody used in our Sumoylation Control kits. Sumoylation Protocol: 1. Prepare a suitable purified substrate protein. (For the control, use 2µl Topoisomerase I marker for each reaction.) 2. In each reaction, add 4µl E2 to substrate first, then 2µl Sumo 1, 2µl 10x reaction buffer, 2µl E1. Finally, add H2O to bring up to 20µl. We would recommend adding fresh 2mM ATP to be sure that sufficient energy is supplied. 3. The best reaction concentration of proteins is as following: Aos 1 + Uba 2: 7.5µg/ml. Ubc 9: 50µg/ml. SUMO 1: 50µg/ml. For the control assay we recommend running the assay at 37ºC for 30-60 minutes. 4. Detect the reaction products by Western blot using a suitable antibody. For the control reaction use 1/1000 dilution of the supplied Topoisomerase I antibody. Four sumoylated bands should be seen on the gel for the control reaction. This assay has been shown to work with crude extracts. Be aware that Uba 2 contains his-rich regions which might cross-react with antibodies against the 6x-His epitope tag. During western analysis with anti-6x-His antibodies, Uba 2 at 80 kDa might be shown.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
Purity	Whole antiserum
Primary antibody notes	This product can be used as part of an assay for sumoylation activity. Human Aos 1 + Uba 2

(ab3804), Ubc 9 (ab3803) and Sumo 1 (ab3801) can be used to promote in vitro sumoylation of a sumoylation marker (human Topoisomerase I protein fragment) (ab3828). The reaction products can be detected using our Sumo 1 (ab3819 and ab3824) and Topoisomerase I (ab3825) antibodies. Sumoylation assays are carried out in a final volume of 20µl in reaction conditions (20 mM Hepes pH 7.5, 5mM MgCl2, 2mM ATP). This antibody is equivalent to the antibody used in our Sumoylation Control kits. Sumoylation Protocol: 1. Prepare a suitable purified substrate protein. (For the control, use 2µl Topoisomerase I marker for each reaction.) 2. In each reaction, add 4µl E2 to substrate first, then 2µl Sumo 1, 2µl 10x reaction buffer, 2µl E1. Finally, add H2O to bring up to 20µl. We would recommend adding fresh 2mM ATP to be sure that sufficient energy is supplied. 3. The best reaction concentration of proteins is as following: Aos 1 + Uba 2: 7.5µg/ml. Ubc 9: 50µg/ml. SUMO 1: 50µg/ml. For the control assay we recommend running the assay at 37ºC for 30-60 minutes. 4. Detect the reaction products by Western blot using a suitable antibody. For the control reaction use 1/1000 dilution of the supplied Topoisomerase I antibody. Four sumoylated bands should be seen on the gel for the control reaction. This assay has been shown to work with crude extracts. Be aware that Uba 2 contains his-rich regions which might cross-react with antibodies against the 6x-His epitope tag. During western analysis with anti-6x-His antibodies, Uba 2 at 80 kDa might be shown.

Clonality	Polyclonal
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab3825** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

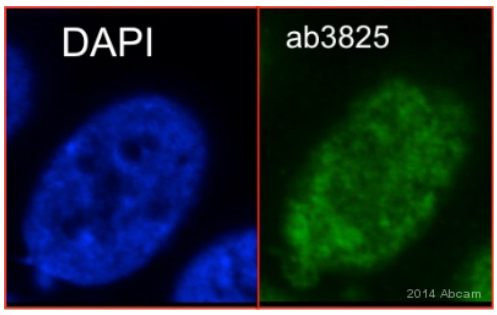
Application	Abreviews	Notes
ICC/IF	★★★★☆	Use at an assay dependent concentration. PubMed: 20655466
IHC-Fr		Use at an assay dependent concentration.
WB	★★★★☆	1/500 - 1/1000. Detects a band of approximately 92 kDa (predicted molecular weight: 91 kDa).
IP	★★★★☆	Use at an assay dependent concentration. PubMed: 17290216
ChIP		Use at an assay dependent concentration. PubMed: 20655466

Target

Function	The reaction catalyzed by topoisomerases leads to the conversion of one topological isomer of DNA to another.
Involvement in disease	Note=A chromosomal aberration involving TOP1 is found in a form of therapy-related myelodysplastic syndrome. Translocation t(11;20)(p15;q11) with NUP98.
Sequence similarities	Belongs to the eukaryotic type I topoisomerase family.
Post-translational modifications	Sumoylated. Lys-117 is the main site of sumoylation. Sumoylation plays a role in partitioning TOP1 between nucleoli and nucleoplasm. Levels are dramatically increased on camptothecin (CPT) treatment.
Cellular localization	Nucleus > nucleolus. Nucleus > nucleoplasm. Diffuse nuclear localization with some enrichment in

nucleoli. On CPT treatment, cleared from nucleoli into nucleoplasm. Sumolyated forms found in both nucleoplasm and nucleoli.

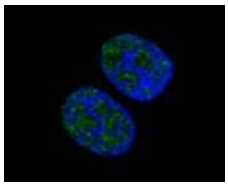
Images



Immunocytochemistry/ Immunofluorescence - Anti-Topoisomerase I antibody - ChIP Grade (ab3825)

This image is courtesy of an anonymous Abreview

ab3825 staining Topoisomerase I in human 293T cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde, permeabilized with 0.5% Triton X-100 and blocked with 3% BSA for 1 hour at 22°C. Samples were incubated with primary antibody (1/100 in PBS + 1% BSA) for 1 hour at 22°C. An Alexa Fluor® 488-conjugated donkey anti-rabbit IgG polyclonal (1/1000) was used as the secondary antibody.

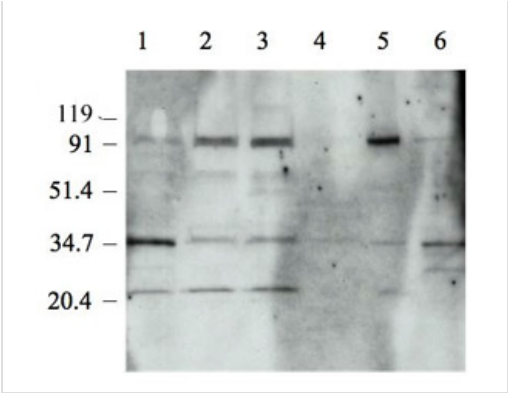


Immunocytochemistry/ Immunofluorescence - Anti-Topoisomerase I antibody - ChIP Grade (ab3825)

blue: DAPI; green: Topo I. Hela cell staining.

Western blot - Anti-Topoisomerase I antibody - ChIP Grade (ab3825)

Primary antibody incubated at a 1/1000 dilution for 60 minutes at 25ºC.



Western blot - Anti-Topoisomerase I antibody - ChIP Grade (ab3825)

All lanes : Anti-Topoisomerase I antibody - ChIP Grade (ab3825) at 1/5000 dilution

Lane 1 : SW210.5 human SCLC cell xenograft lysate.
Lane 2 : 5M2 human NSCLC cell lysate (bicarb. buffer).
Lane 3 : 5M2 human NSCLC cell lysate (M-Per buffer).
Lane 4 : Normal human lung tissue lysate.
Lane 5 : A431 cell (human epidermoid carcinoma) lysate .
Lane 6 : Rh30 cell (human rhabdomyosarcoma) lysate.

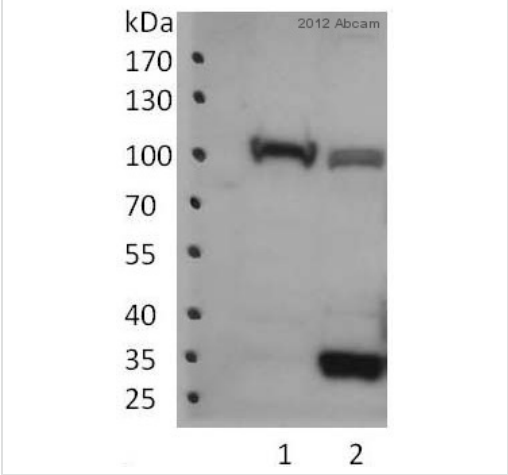
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti rabbit heavy and light (HRP) at 1/25000 dilution

Performed under reducing conditions.

Predicted band size: 91 kDa

This image is courtesy of an Abreview submitted by **Mike Campa** on **7 September 2005**. We do not have any further information relating to this image.



Western blot - Anti-Topoisomerase I antibody - ChIP Grade (ab3825)
This image is courtesy of an anonymous Abreview

All lanes : Anti-Topoisomerase I antibody - ChIP Grade (ab3825) at 1/200 dilution

Lane 1 : K562 nuclear lysate
Lane 2 : Mouse N2a whole cell lysate at 100 µg

Secondary
All lanes : Donkey anti-rabbit IgG HRP polyclonal at 1/1000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 91 kDa
Observed band size: 100 kDa
why is the actual band size different from the predicted?
Additional bands at: 35 kDa (possible non-specific binding)

Exposure time: 15 seconds

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