

Product datasheet

Anti-Topoisomerase I antibody - ChIP Grade ab3825

★★★★☆ 9 Abreviews 19 References 5 Images

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	<p>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 37°C 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.</p>

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 37°C 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.

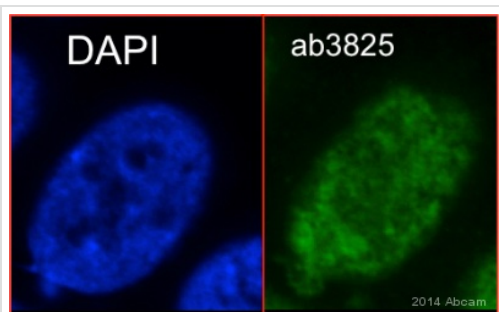
Post-translational modifications

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



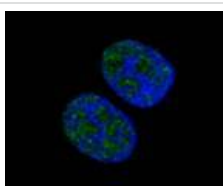
Immunocytochemistry/ Immunofluorescence - Anti-Topoisomerase I antibody - ChIP Grade (ab3825)
This image is courtesy of an anonymous Abreview

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.



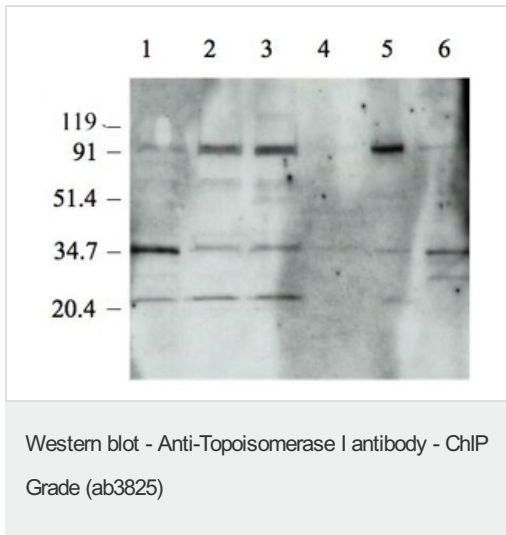
Immunocytochemistry/ Immunofluorescence - Anti-Topoisomerase I antibody - ChIP Grade (ab3825)

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 25°C.



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.

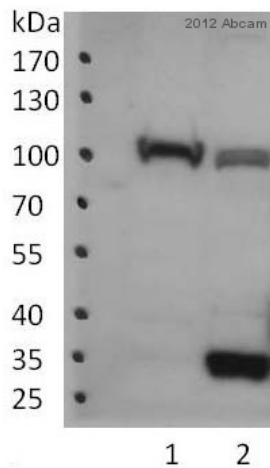
Secondary

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 μ g

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

Additional bands at: 35 kDa (possible non-specific binding)

Exposure time: 15 seconds

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