

Product datasheet

Recombinant Human Topoisomerase I protein ab3828

Overview

Product name	Recombinant Human Topoisomerase I protein
Protein length	Protein fragment

Description

Nature	Recombinant
Source	Escherichia coli

Amino Acid Sequence

Accession	P11387
Species	Human
Molecular weight	91 kDa
Amino acids	1 to 200

Specifications

Our [Abpromise guarantee](#) covers the use of **ab3828** in the following tested applications.

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

Form	Liquid
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Additional notes	<p>Partial fragment of Topo I protein for sumoylation purposes. This fragment has no enzymatic activity. The final fraction of protein contains a single polypeptide band of 50 kDa and is devoid of contaminating nuclease activity.</p> <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). 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</p>
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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.

Enough Topo I marker fragment is supplied for 20 standard assays as described in the protocol above.

Preparation and Storage

Stability and Storage 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.

Constituent: 20% Glycerol

General Info

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. Sumoylated forms found in both nucleoplasm and nucleoli.

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