abcam

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
Additional notes	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. 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 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.

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.

PBS with 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. Sumolyated forms found in both nucleoplasm and nucleoli.

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