Product datasheet

Anti-Topoisomerase I antibody ab85038

Overview

Product name: Anti-Topoisomerase I antibody
Description: Rabbit polyclonal to Topoisomerase I
Host species: Rabbit
Tested applications: Suitable for: ICC/IF, WB, IHC-P, IP
Species reactivity
Reacts with: Mouse, Rat, Human
Predicted to work with: Chicken, Hamster, Cow, Monkey

Immunogen: Synthetic peptide corresponding to Human Topoisomerase I aa 1-100 (N terminal) conjugated to keyhole limpet haemocyanin.
(Peptide available as ab94391)

Positive control: This antibody gave a positive signal in the following human whole cell lysates: HeLa; Jurkat; HepG2; HEK293; MCF7; Caco2; SHSY5Y; NIH3T3; PC12.

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.
Storage buffer: Preservative: 0.02% Sodium Azide
Constituents: 1% BSA, PBS, pH 7.4

Purity: Immunogen affinity purified
Clonality: Polyclonal
Isotype: IgG

Applications

Our Abpromise guarantee covers the use of ab85038 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.
**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.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICC/IF</td>
<td></td>
<td>Use a concentration of 1 µg/ml.</td>
</tr>
<tr>
<td>WB</td>
<td></td>
<td>Use a concentration of 1 µg/ml. Detects a band of approximately 90 kDa (predicted molecular weight: 90 kDa).</td>
</tr>
<tr>
<td>IHC-P</td>
<td></td>
<td>Use a concentration of 1 µg/ml.</td>
</tr>
<tr>
<td>IP</td>
<td></td>
<td>Use a concentration of 5 µg/ml.</td>
</tr>
</tbody>
</table>

**Target**

**Images**

**All lanes**: Anti-Topoisomerase I antibody (ab85038) at 1 µg/ml

- **Lane 1**: HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
- **Lane 2**: Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate
- **Lane 3**: HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate
- **Lane 4**: HEK293 (Human embryonic kidney carcinoma cell line) Whole Cell Lysate
- **Lane 5**: MCF7 (Human breast adenocarcinoma cell line) Whole Cell Lysate
- **Lane 6**: Caco 2 (Human colonic carcinoma cell line) Whole Cell Lysate
- **Lane 7**: SHSY-5Y (Human neuroblastoma cell line) Whole Cell Lysate

[Western blot - Anti-Topoisomerase I antibody (ab85038)]
Lysates/proteins at 10 µg per lane.

**Secondary**

**All lanes**: Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size**: 90 kDa

**Observed band size**: 90 kDa

**Exposure time**: 30 seconds

Human DNA topoisomerase 1 contains a potential sumoylation site (SwissProt) which may explain its migration at a higher molecular weight than predicted.

---

**Western blot - Anti-Topoisomerase I antibody (ab85038)**

**All lanes**: Anti-Topoisomerase I antibody (ab85038) at 1 µg/ml

**Lane 1**: NIH 3T3 (Mouse embryonic fibroblast cell line) Whole Cell Lysate

**Lane 2**: PC12 (Rat adrenal pheochromocytoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

**Secondary**

**All lanes**: Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (ab97080) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size**: 90 kDa

**Observed band size**: 100 kDa

Why is the actual band size different from the predicted?

**Additional bands at**: 50 kDa, 55 kDa, 70 kDa. We are unsure as to the identity of these extra bands.
Immunoprecipitation - Anti-Topoisomerase I antibody

**Topoisomerase I** was immunoprecipitated using 0.5mg Hek293 whole cell extract, 5µg of Rabbit polyclonal to Topoisomerase I and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

The antibody was incubated under agitation with Protein G beads for 10min, Hek293 whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab85038.


Band: 90kDa; Topoisomerase I

Immunocytochemistry/ Immunofluorescence - Anti-Topoisomerase I antibody (ab85038)

ICC/IF image of ab85038 stained HeLa cells. The cells were 4% PFA fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab85038, 1µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM. This antibody also gave a positive result in 4% PFA fixed (10 min) Hek293, HepG2 and MCF7 cells at 1µg/ml, and in 100% methanol fixed (5 min) HeLa cells at 1µg/ml.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards
If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors