Product name: Anti-Topoisomerase I antibody [EPR5375] ab109374

Description: Rabbit monoclonal [EPR5375] to Topoisomerase I

Host species: Rabbit

Tested applications: Suitable for: WB, IHC-P, Flow Cyt, ICC/IF

Unsuitable for: IP

Species reactivity: Reacts with: Mouse, Rat, Human

Immunogen: Synthetic peptide within Human Topoisomerase I aa 1-100 (N terminal). The exact sequence is proprietary.

Positive control: WB; Jurkat, SW480, MCF7, HepG2, Neuro-2a, PC-12, and K562 cell lysates; IHC-P: Human breast carcinoma, Human colonic carcinoma tissues, Human clear cell carcinoma of kidney and Mouse kidney, and Rat colon tissue; ICC/IF: MCF7 cells;

General notes:

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

This product is a recombinant rabbit monoclonal antibody.

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. Stable for 12 months at -20°C.

Storage buffer: pH: 7.20
Preservative: 0.01% Sodium azide
Constituents: PBS, 40% Glycerol, 0.05% BSA
Purity  Protein A purified
Clonality  Monoclonal
Clone number  EPR5375
Isotype  IgG

Applications

Our Abpromise guarantee covers the use of ab109374 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tr>
<td>IHC-P</td>
<td>1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.</td>
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<tr>
<td>Flow Cyt</td>
<td>1/10 - 1/100. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.</td>
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<tr>
<td>ICC/IF</td>
<td>1/500. For unpurified use at 1/100 - 1/250</td>
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Application notes  Is unsuitable for IP.

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 nucleol.

Images
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Rat colon tissue sections labeling Topoisomerase I with Purified ab109374 at 1:100 dilution (1.29 µg/ml). Heat mediated antigen retrieval was performed by performing heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). ImmunoHistoprobe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.

Immunocytochemistry/ Immunofluorescence analysis of MCF7 (Human breast adenocarcinoma epithelial cell) cells labeling Topoisomerase I with Purified ab109374 at 1/500 dilution (0.3 µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1/1000 (2 µg/ml) dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.
Flow Cytometry analysis of HepG2 (Human hepatocellular carcinoma epithelial cell) cells labeling Topoisomerase I with Purified ab109374 at 1/20 dilution (10 µg/ml) (red). Cells were fixed with 80% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Mouse kidney tissue sections labeling Topoisomerase I with Purified ab109374 at 1:100 dilution (1.29 µg/ml). Heat mediated antigen retrieval was performed Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control:PBS instead of the primary antibody. Hematoxylin was used as a counterstain.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human clear cell carcinoma of kidney tissue sections labeling Topoisomerase I with Purified ab109374 at 1:100 dilution (1.29 µg/ml). Heat mediated antigen retrieval was performed. Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). ImmunohistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.
Overlay histogram showing HepG2 cells stained with ab109374 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab109374, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1μg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.
Western blot - Anti-Topoisomerase I antibody [EPR5375] (ab109374)

**All lanes**: Anti-Topoisomerase I antibody [EPR5375] (ab109374) at 1/10000 dilution

- **Lane 1**: Jurkat cell lysate
- **Lane 2**: MCF7 cell lysate
- **Lane 3**: SW480 cell lysate
- **Lane 4**: K562 cell lysate

Lysates/proteins at 10 µg per lane.

**Predicted band size**: 91 kDa

Immunohistochemical analysis of paraffin-embedded Human breast carcinoma tissue using ab109374 at a dilution of 1/100.

Immunohistochemical analysis of paraffin-embedded Human colonic carcinoma tissue using ab109374 at a dilution of 1/100.
Immunofluorescent staining of MCF7 cells using ab109374 at a dilution of 1/100.

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

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