# abcam

# Product datasheet

# Anti-Topoisomerase I antibody [EPR5375] ab109374



★★★★★ 8 Abreviews 36 References 13 Images

#### Overview

**Product name** Anti-Topoisomerase I antibody [EPR5375]

**Description** Rabbit monoclonal [EPR5375] to Topoisomerase I

**Host species** Rabbit

**Tested applications** Suitable for: Flow Cyt (Intra), WB, IHC-P, ICC/IF

Unsuitable for: IP

Species reactivity Reacts with: Mouse. Rat. Human

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

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 This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

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

#### **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**

The Abpromise guarantee Our <u>Abpromise guarantee</u> 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.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/10 - 1/100. <b>ab172730</b> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB	*** <u>*</u>	1/10000 - 1/50000. Predicted molecular weight: 91 kDa.
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.  See IHC antigen retrieval protocols.
ICC/IF	★★★★☆ (1)	1/500. For unpurified use at 1/100 - 1/250

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

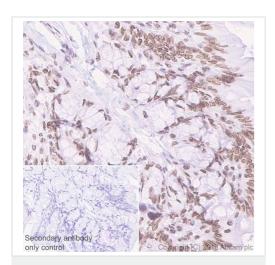
(CPT) treatment.

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

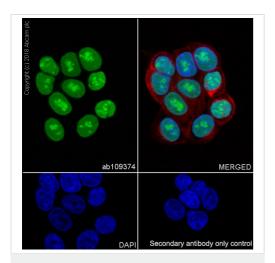
both nucleoplasm and nucleoli.

# **Images**



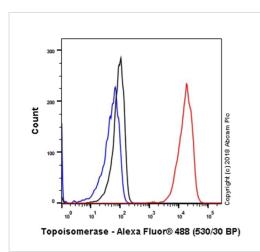
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Topoisomerase I antibody [EPR5375] (ab109374)

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 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.



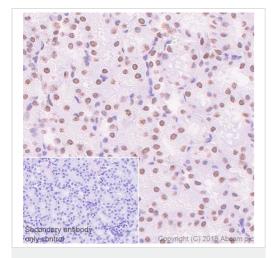
Immunocytochemistry/ Immunofluorescence - Anti-Topoisomerase I antibody [EPR5375] (ab109374)

Immunocytochemistry/ Immunofluorescence analysis of MCF7 (Human breast adenocarcinoma epithelial cell) cells labeling Topoisomerase I with Purified ab109374 at 1/500 dilution (0.3  $\mu$ g/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  $\mu$ g/ml). Goat anti rabbit lgG (Alexa Fluor® 488, <u>ab150077</u>) was used as the secondary antibody at 1/1000 (2  $\mu$ g/ml) dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



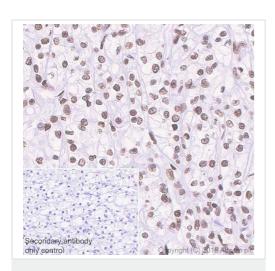
Flow Cytometry (Intracellular) - Anti-Topoisomerase I antibody [EPR5375] (ab109374)

Intracellular Flow Cytometry analysis of HepG2 (Human hepatocellular carcinoma epithelial cell) cells labeling Topoisomerase I with Purified ab109374 at 1/20 dilution (10µg/ml) (red). Cells were fixed with 80% Methanol. A Goat anti rabbit lgG (Alexa Fluorr<sup>®</sup> 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal lgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



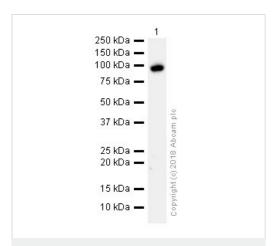
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Topoisomerase I antibody [EPR5375] (ab109374)

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 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 paraffinembedded sections) - Anti-Topoisomerase I antibody [EPR5375] (ab109374)

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 using <a href="mailto:ab93684">ab93684</a> (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.



Western blot - Anti-Topoisomerase I antibody [EPR5375] (ab109374)

Anti-Topoisomerase I antibody [EPR5375] (ab109374) at 1/50000 dilution (Purified) + PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysates at 20  $\mu g$ 

#### **Secondary**

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

**Predicted band size:** 91 kDa **Observed band size:** 91 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST



[EPR5375] (ab109374)

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

**Lane 1 :** MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysates

**Lane 2**: Jurkat (Human T cell leukemia T lymphocyte) whole cell lysates

**Lane 3**: HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysates

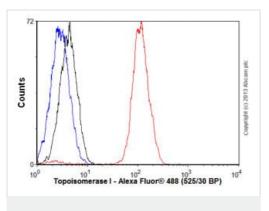
**Lane 4 :** Neuro-2a (Mouse neuroblastoma neuroblast) whole cell lysates

Lysates/proteins at 20 µg per lane.

# Secondary

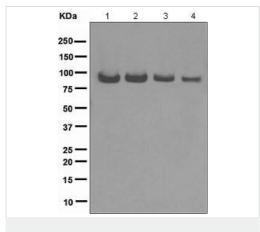
**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

**Predicted band size:** 91 kDa **Observed band size:** 91 kDa

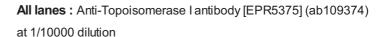


Flow Cytometry (Intracellular) - Anti-Topoisomerase I antibody [EPR5375] (ab109374)

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 Fluorr® 488 goat anti-rabbit lgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (1µg/1x106 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.



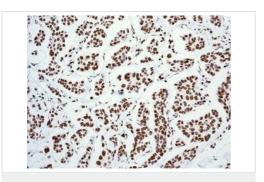
Western blot - Anti-Topoisomerase I antibody [EPR5375] (ab109374)



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



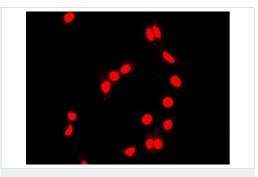
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Topoisomerase I antibody [EPR5375] (ab109374)

Immunohistochemical analysis of paraffin-embedded Human breast carcinoma tissue using ab109374 at a dilution of 1/100. Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.

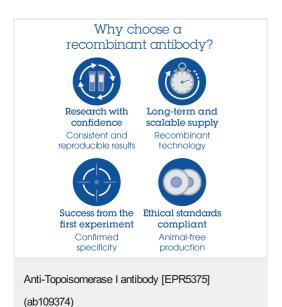


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Topoisomerase I antibody [EPR5375] (ab109374)

Immunohistochemical analysis of paraffin-embedded Human colonic carcinoma tissue using ab109374 at a dilution of 1/100. Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-Topoisomerase I antibody [EPR5375] (ab109374) Immunofluorescent staining of MCF7 cells using ab109374 at a dilution of 1/100.



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