

## Technical Data Sheet

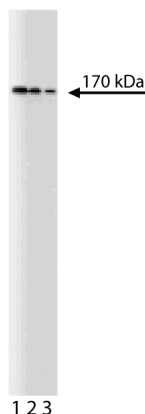
**Purified Mouse Anti- Human Topo II $\alpha$** **Product Information**

<b>Material Number:</b>	611326
<b>Size:</b>	50 $\mu$ g
<b>Concentration:</b>	250 $\mu$ g/ml
<b>Clone:</b>	31/Topo II $\alpha$
<b>Immunogen:</b>	Human Topo II $\alpha$ aa. 1245-1361
<b>Isotype:</b>	Mouse IgG1
<b>Reactivity:</b>	QC Testing: Human
<b>Target MW:</b>	170 kDa
<b>Storage Buffer:</b>	Aqueous buffered solution containing BSA, glycerol, and $\leq 0.09\%$ sodium azide.

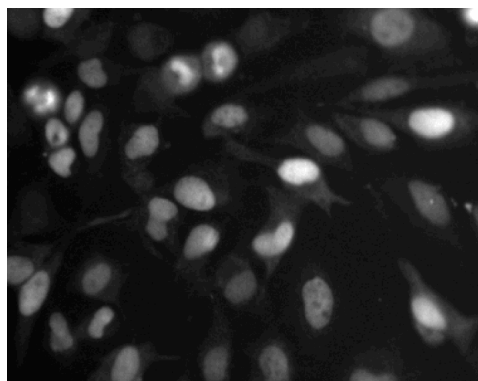
**Description**

Eukaryotic DNA topoisomerase II, a ubiquitous ATP-dependent type II topoisomerase, is an essential nuclear enzyme in DNA replication and transcription, chromatin segregation, and cell cycle progression. These enzymes transiently break a pair of complementary strands in double-stranded DNA to form a gate for the passage of duplex DNA. Two isoforms of DNA topoisomerase II have been identified, topo II $\alpha$  and topo II $\beta$ . These have a high degree of homology, except for some divergence in the C-terminal region. Both contain multiple bipartite nuclear localization sequences (NLS) that mediate their subnuclear localization. Topo II $\alpha$  levels rise during late S phase and peak in G2-M, whereas topo II $\beta$  levels remain constant throughout the cell cycle. In addition, topo II $\alpha$  is expressed in proliferating cells, while topo II $\beta$  is expressed in many tissues. The exact role of these two isoforms during cell proliferation is not known, however the differences in cellular expression implicate different physiological roles. Both isoforms may also be important targets for anticancer agents that exert cytotoxicity in proliferating cells via stabilization of a topo II-DNA complex.

This antibody is routinely tested by Western blot analysis and immunofluorescent imaging. Other applications were tested at BD Biosciences Pharmingen during antibody development only.



**Western blot analysis of Topo II $\alpha$  on a HeLa lysate.**  
Lane 1: 1:500, lane 2: 1:1000, lane 3: 1:2000 dilution of the Topo II $\alpha$  antibody.



**Immunofluorescent staining of HeLa cells.** Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~10 000 cells per well. After overnight incubation, cells were stained using the methanol fix/perm protocol (see Recommended Assay Procedure) and the anti-Topo II $\alpha$  antibody. The second step reagent was FITC goat anti mouse Ig (Cat. No. 554001). The image was taken on a Pathway 850 imager using a 20x objective. This antibody also stained A549 and U2OS cells and can be used with either fix/perm protocol (see Recommended Assay Procedure).

**BD Biosciences**

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.259.0187	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

For country-specific contact information, visit [bdbiosciences.com/how\\_to\\_order/](http://bdbiosciences.com/how_to_order/)

Conditions: The information disclosed herein is not to be construed as a recommendation to use the above product in violation of any patents. BD Biosciences will not be held responsible for patent infringement or other violations that may occur with the use of our products. Purchase does not include or carry any right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Becton Dickinson and Company is strictly prohibited.

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale.

BD, BD Logo and all other trademarks are the property of Becton, Dickinson and Company. ©2006 BD

**BD****BD Biosciences**

## Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Store undiluted at -20° C.

## Application Notes

### Application

Bioimaging	Routinely Tested
Western blot	Routinely Tested
Immunofluorescence	Tested During Development

### Recommended Assay Procedure:

#### Methanol Procedure for a 96 well plate:

Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 90% methanol. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Wash three times with PBS. Flick out PBS and add second step reagent. Incubate for 1 hour at RT. Wash three times with PBS. Image sample.

#### Triton-X 100 Procedure for a 96 well plate:

Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 0.1% Triton-X 100. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Flick out and wash three times with PBS. Flick out and add second step reagent. Incubate for 1 hour at RT. Flick out and wash three times with PBS. Image sample.

## Suggested Companion Products

Catalog Number	Name	Size	Clone
611449	HeLa Cell Lysate	500 µg	(none)
554002	HRP Goat Anti-Mouse Igs	1.0 ml	(none)
554001	FITC Goat Anti-Mouse Igs	0.5 mg	Polyclonal

## Product Notices

1. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
2. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. Please refer to [www.bdbiosciences.com/pharmingen/protocols](http://www.bdbiosciences.com/pharmingen/protocols) for technical protocols.

## References

Brown MS, Holden JA, Rahn MP, Perkins SL. Immunohistochemical staining for DNA topoisomerase IIa in Hodgkin's disease. *Am J Clin Pathol.* 1998; 109(1):39-44.(Biology)  
Herzog CE, Holmes KA, Tuschong LM, Ganapathi R, Zwelling LA. Absence of topoisomerase IIbeta in an amsacrine-resistant human leukemia cell line with mutant topoisomerase IIalpha. *Cancer Res.* 1998; 58(23):5298-5300.(Biology)  
Mirski SE, Gerlach JH, Cole SP. Sequence determinants of nuclear localization in the alpha and beta isoforms of human topoisomerase II. *Exp Cell Res.* 1999; 251(2):329-339.(Biology)  
Tsai-Pflugfelder M, Liu LF, Liu AA, et al. Cloning and sequencing of cDNA encoding human DNA topoisomerase II and localization of the gene to chromosome region 17q21-22. *Proc Natl Acad Sci U S A.* 1988; 85(19):7177-7181.(Biology)