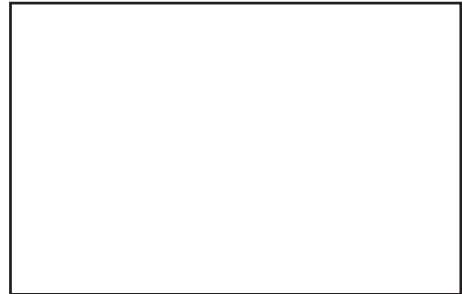


# *Purified Human DNA Topoisomerase II $\alpha$ (p170 form)*

*Catalog No. 2000H-1 [250 units]*

*Catalog No. 2000H-2 [500 units]*

## Product Description



Contains purified DNA Topoisomerase II (p170) over expressed and purified to homogeneity to a single band on SDS-PAGE. Included with the enzyme are two buffers (Incomplete Buffer A and ATP Buffer B) that are used to make a final working stock of 5x topo II assay buffer, as described below.

## Storage and Shipping Conditions

The enzyme is shipped on dry ice and should be stored at -70° C. We also recommend that the enzyme be aliquoted after the first thaw (repeated rounds of freeze/thaw will lead to loss of activity); the enzyme activity is stable for 1-2 days (not longer) at 4°C or on ice. The 10x Incomplete Buffer A and the 10x ATP Buffer B should be stored frozen at -20°C.

*IMPORTANT NOTE REGARDING ACTIVITY:* The enzyme is provided at a unit/ul concentration that represents a certified minimum. For example, we certify that the product will have X units/ul under conditions of our assay. In some cases, activity may be greater than X units to take into account that freezing/thawing may lead to some loss over time. The enzyme will retain the certified minimum unit concentration (see label) for 6 months after receipt.

## Unit Definition

One unit of Topoisomerase II can decatenate 0.2 ug (200 ng) of KDNA in 30 min at 37°C. Note that relaxation activity (with a plasmid) is generally about 20-40% of the decatention units (kDNA is a better substrate).

### Product Application and Disclaimer

This product is not licensed or approved for administration to humans or animals. It may be used with experimental animals only. The product is for in vitro research diagnostic studies only. The product is non-infectious and non-hazardous to human health. This information is based on present knowledge and does not constitute a guarantee for any specific product features and shall not establish a legally valid contractual relationship. TopoGEN, Inc. shall not be held liable for product failure due to mishandling and incorrect storage by end user. TopoGEN's liability is limited to credit or product replacement.



## Human Topoisomerase II $\alpha$ Quality Control Tests: Catalog #2000H

1. Nuclease contamination was assayed by testing for the formation of linear KDNA and linear plasmid DNA. Incubation of 1  $\mu$ g of catenated KDNA or supercoiled pUC19 DNA was performed for 4 hrs. at 37° (under topo II assay conditions and with or without ATP). Linear DNA was not generated under these conditions.
2. Cross contamination with topo I was assessed by assaying for relaxation of supercoiled DNA under conditions optimal for topo I activity. Excess purified topo II was incubated with pRYG DNA in the absence of ATP (with and without Mg<sup>++</sup>) for 2 hr at 37° C. Under these conditions, relaxation of pRYG supercoiled DNA was not seen.
3. A single band is seen when loaded on an SDS-PAGE gel (170kDa). No other bands are detectable in an overloaded gel. Protein concentrations typically vary from lot to lot between 25- and 60 ug/ml.
4. The final fraction of topoisomerase II comes off an FPLC Superose 6 column in the following buffer: 10% glycerol, 50 mM Tris (pH 7.7), 1 mM EDTA and EGTA, 260 mM NaCl.

## Dilution Buffer

The enzyme should not be diluted for cleavage assays since larger amounts of enzyme activity are required (typically 2-6 units per reaction). For catalytic assays (decatenation or relaxation assays), dilutions should be performed in a dilution buffer (10 mM Tris-Cl (pH 7.5), 500 mM NaCl or KCl, 1 mM PMSF and 2 mM DTT and 50 ug BSA/ml). Note that very large dilutions of the enzyme (greater 10 fold) may accelerate the decay of enzyme activity.

## Assay Conditions

Catalytic assays (decatenation) are carried out using kinetoplast DNA substrate in a final volume of 20-30  $\mu$ l in topo II reaction buffer (1x topo II buffer = 50 mM Tris-Cl, pH 8.0, 150 mM NaCl, 10 mM MgCl<sub>2</sub>, 5 mM ATP 0.5 mM dithiothreitol and 30 ug BSA/ml) with 0.2  $\mu$ g KDNA. Reactions are terminated with 5x stop buffer (5  $\mu$ l per 20  $\mu$ l reaction volume). Stop buffer contains 5% sarkosyl, 0.0025% bromophenol blue, 25% glycerol. Reaction products are analyzed on a 1% agarose gel containing 0.5 ug/ml ethidium bromide. Resolution of a 2.5 kb DNA minicircle decatenated product can be easily monitored with a hand held UV light source while the gel is running. Gels can be at a very high voltage since resolution of decatenation products is easy to achieve.

**TopoGEN provides two buffers that \*must be mixed in equal proportions to yield a final 5x working stock of assay buffer. The 5x Complete Buffer should be made fresh daily!**

1. Incomplete **Buffer A** contains the following: 0.5M Tris-HCl (pH8.0), 1.5M NaCl, 0.1M MgCl<sub>2</sub>, 5 mM dithiothreitol, 300 ug/ml BSA). [0.25 mL provided]
2. Incomplete ATP **Buffer B** contains 20 mM ATP in water [0.25 mL provided].

*\*These two buffers must be made fresh and mixed in equal volumes to make the final 5x Complete Topo II Assay Buffer (A+B) as follows.* Add equal volume of Buffer A and Buffer B to give a final 5x Topo II assay buffer fresh and ready to use. Prepare only as much of the Complete 5x Topo II Assay Buffer (A+B) as needed for each day or set of experiments. For example: If you are performing 10 assays (each with a final volume of say 20 ul per assay) in a single day, you will need 40 ul of the 5x Complete Buffer (4 ul/reaction x 10 reactions). Thus, you should prepare slightly more than needed as follows: 25 ul of Buffer A plus 25 ul of B to give 50 ul total of Complete Buffer (5x stock). Note that this buffer should be kept on ice and used the same day, then discarded.

## References

- Muller et al., Biochemistry 27: 8369-8379 (1988)  
Spitzner and Muller, Nuc. Acid. Res. 16: 5533-5556 (1988)

