TopoGEN Products and Services for **Cancer Research.**

Purified Human DNA Topoisomerase IIα

Product Description

Contains purified DNA Topoisomerase II (p170, over- expressed and purified). 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 generally 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 will decatenate 0.2 ug of kDNA in 30 min at 37°C. We do NOT recommend using a plasmid DNA substrate with this product for several reasons, the most important of of which is our unit definition is based on kDNA. TopoGEN's Topoisomerase II is validated and is pre-screened based on kDNA units (a natural and optimal substrate). We do not recommend using 'relaxation' with plasmid DNA because supercoiled DNA substrates (especially from other suppliers or made in-house) may carry over contaminants (heavy metals in the ion atmosphere of some plasmid preps) which may inhibit enzyme activity. Finally, most plasmids are a mix of supercoiled (circular DNA) and nicked DNA. This contaminating nicked plasmid DNA may significantly affect quantifying enzyme action. For these reasons, we cannot certify our Topoisomerase IIa based on supercoiling/relaxation assays using plasmids.

Topo IIα Quality Control Tests

1. Nuclease contamination was assayed by testing for the formation of linear kDNA. Incubation of 1 µg of catenated kDNA is peformed for 4 hrs. at 37°C (under topo II assay conditions and with or without ATP). Linear DNA is not generated under these conditions.
2. A major band is seen when loaded on an SDS--PAGE gel (170kDa). Protein concentrations typically vary from lot to lot between 25-- and 60 ug/ml.
4. The final fraction of topoisomerase II comes off the purification column in the following buffer: 10% glycerol, 50 mM Tris (pH 7.7), 1 mM EDTA and EGTA, 650 mM NaCl.

Dilution Buffer

The enzyme should not be diluted for cleavage assays since larger amounts of enyzme 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 (>5-10 fold) may accelerate the decay of enzyme activity

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Assay Conditions

Catalytic assays (decatenation) are carried out using kinetoplast DNA substrate in a final volume of 20--30 µl in topo II reaction buffer (1x topo II buffer = 50 mM Tris--Cl, pH 8.0, 150 mM NaCl, 10 mM MgCl2. 5 mM ATP 0.5 mM dithiothreitol and 30 ug BSA/ml) with 0.2 µg KDNA. Reactions are terminated with 5x stop buffer (5 µl per 20 µl 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 or other DNA stain. Resolution of a 2.5 kb kDNA 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 decatenation products are easily resolved.

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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), 1.5 M NaCl, 0.1M MgCl2, 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)