

Human DNA Topoisomerase alpha (p170) Cat#2000H

Description:

We purify the alpha form of topo II using a proprietary method developed by the staff at TopoGEN. The purified enzyme is completely free of topoisomerase I contamination and nucleases. It alters linking number of a unique topoisomer in steps of two and the major polypeptide detected on SDS-PAGE is 170 kDa; no other bands are visible on an overloaded gel.

Quality Control Tests:

Nuclease contamination: assayed by testing for linear KDNA and linear plasmid DNA formation. Incubation of 1 µg of catenated KDNA or supercoiled pUC19 DNA for 4 hrs. at 37° (under topo II assay conditions and with or without ATP).

Cross contamination with topo I was assessed by assaying for relaxation of supercoiled DNA under conditions where topo II activity is suppressed. Excess purified topo II was incubated with pRYG DNA in the absence of ATP (with and without Mg⁺⁺) for 2 hr at 37° C. Under these conditions, relaxation of pRYG supercoiled DNA must not be detected.

A single band of 170 kDa was seen when an SDS-PAGE gel was overloaded with the pure protein. There is no 180 kDa Topo II form in the final fraction. (Protein concentration is variable from lot to lot but usually is in the range of 5-50 ug per ml.) Usually the enzyme is supplied at 2 units/ul (1 unit will decatenate 0.2 ug of KDNA in 30 min at 37°C.)

The final fraction of topoisomerase II comes off an FPLC column in the following buffer: 25% glycerol, 50 mM potassium phosphate, pH 7.1, 0.5 mM DTT, 0.1 mM EDTA, 350 mM NaCl.

Assay Conditions:

Decatenation assays are carried out using kinetoplast DNA substrate in a final volume of 20-30 µl in topo II reaction buffer (50 mM Tris-Cl, pH 8.0, 120 mM KCl, 10 mM MgCl₂, 0.5 mM ATP, 0.5 mM dithiothreitol) with 0.2 µg KDNA. A *10X assay buffer is included with the enzyme. Reactions are terminated with 5 µl (per 20 µl reaction volume) of stop buffer (5% sarkosyl, 0.0025% bromophenol blue, 25% glycerol). Reaction products are analyzed on a 1% agarose gel containing 0.5 µg/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.

*NEW: To improve stability, the 10x Topo II Assay buffer is provided as two components: Buffer A (no ATP) and Buffer B (ATP). The Complete 10x Assay buffer is made fresh each time by combining Buffer A and B.

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) on ice.

REFERENCES:

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