Product Description
Topo IV is a type II topoisomerase that is encoded by two genes parC and parE in E. coli (1). Topo IV is essential for decatenation and chromosomal segregation in E. coli; thus, topo IV tends to prefer intermolecular strand passing (catenating and decatenating reactions) over relaxation reactions. Topo IV is also the target for quinolone--based antibacterial agents which act by subverting the enzyme into a DNA damaging agent. TopoGEN offers purified Topo IV for use in all aspects of drug development and screening assays. The enzyme is very active in decatenating kDNA (TG2013) but will also relax plasmid DNA (it is less robust in relaxation activity). Topo IV is purified according to Peng and Marians. Other purified topoisomerases and antibodies are available from TopoGEN and may be ordered on line at www.topogen.com.

Storage and Shipping Conditions
This enzyme should be stored at -70°C and is stable undiluted for at least 6 months in this concentrated state. The enzyme can be aliquoted on first thawing to minimize damage from multiple freeze thaw cycles.

Unit Definition
One unit will decatenate 0.2 ug of kDNA in 30 min at 37°C under conditions described below.

E. coli Topo IV Quality Control Tests
1. Topo IV was overexpressed and purified using published methods. A single band on SDS--PAGE was detected by CB staining for each subunit. Cross contamination by topo I was assessed by assaying for relaxation of supercoiled DNA under conditions optimized for type I activity. Under these conditions, after 2 hours of incubation with pBR322, no relaxation products were detectable.
2. A test for nuclease contamination was carried out by assaying for the formation of linear kDNA and linear plasmid DNA. Incubations of 1 µg of catenated kDNA or supercoiled pUC19 DNA (4 hrs. at 37 C in the presence of 10 mM MgCl2) were performed. Linear DNA or breakdown products were not generated under these conditions.
3. The subunits are >95% pure based upon SDS--PAGE and certified to be endonuclease free.

Assay Conditions
TopoGEN provides a 2-part buffer that should be mixed immediately before use to prepare a final working stock of the assay buffer:
1. Incomplete Buffer A (minus ATP) contains the following: 200 mM HEPES--KOH [pH8], 500 mM potassium glutamate, 50 mM Magnesium Acetate, 50 mM Dithiothreitol, 250 ug BSA/ml.
2. ATP Buffer B contains 100 mM ATP in water. These two buffers must be mixed before use to make the final 5x Complete Topo IV Assay Buffer (A+B) as follows: add 0.1 volume of Buffer B to 1.0 volume Buffer A. Prepare only as much of the Complete Topo IV Assay Buffer (A+B) as needed since this buffer should be kept on ice and used the same day, then discarded. For example, adding 10ul of Buffer B to 100ul of buffer A will yield a final 5x stock Topo IV Complete Assay Buffer.

Dilution Buffer
If necessary, dilute the enzyme using the following buffer (1x): 40 mM HEPES--KOH [pH 7.6], 150 mM Potassium Glutamate, 1 mM dithiothreitol, 1 mM EDTA, 40% glycerol.

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 in 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.

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