

Purified *E. coli* Topoisomerase IV

- Catalog No. 2000EC-1 [250 units]**
- Catalog No. 2000EC-3 [500 units]**
- Catalog No. 2000EC-5 [1000 units]**



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 (Cat. # 2031-1) but will also relax plasmid DNA (it is less robust in relaxation activity). Topo IV is purified according to Peng and Mariani (2).

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 -20°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 μg of kDNA in 30 min at 37°C under conditions described below.

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.



***E. coli* Topo IV Quality Control Tests: Catalog #2000EC**

1. Topo IV was overexpressed and purified using the published methods (2). 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 pBR233, 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° in the presence of 10 mM MgCl₂) were performed. Linear DNA or breakdown products were not generated under these conditions.

2. The subunits are >95% pure based upon SDS-PAGE and certified to be endonuclease free.

Assay Conditions

TopoGEN provides two buffers that are used to prepare a final 10x working stock of assay buffer:

1. Incomplete **Buffer A** contains the following: 200 mM HEPES-KOH [pH7.6], 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 [50 ul provided].

These two buffers must be mixed before use to make the final 10x 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 for each day or set of experiments. Note that this buffer should be kept on ice and used the same day, then discarded.

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.

References

1. Kato et al. (1990) Cell 63:393-404.
2. Peng, H., and Marians, K. (1999). DNA Topoisomerase Protocols Volume I; page 163 (available from TopoGEN, Cat. # VI, see www.topogen.com)

