E. coli Topoisomerase IV Cleavage Kit

(isolated from *Escherichia coli*)

Product Description (Product Numbers #TCK001 and TCK002)

Topoisomerase IV is prepared by overexpressing the par C and par E subunits in *E. coli* and purifying them by methods adapted from Peng and Marians, 1999. It is supplied as a heterotetramer complex. The enzyme is supplied in Dilution Buffer at a concentration suitable for cleavage assays. The activity varies between lot numbers, please see appropriate Certificate of Analysis for activity (see typical titration below).

Store at -80°C. (Stable for 6 months undiluted) For in-vitro laboratory research use only.

Dilution Buffer

40 mM HEPES-KOH (pH 7.6)
100 mM Potassium Glutamate
1 mM dithiothreitol
1 mM EDTA
40% (v/v) glycerol

Assay Buffer

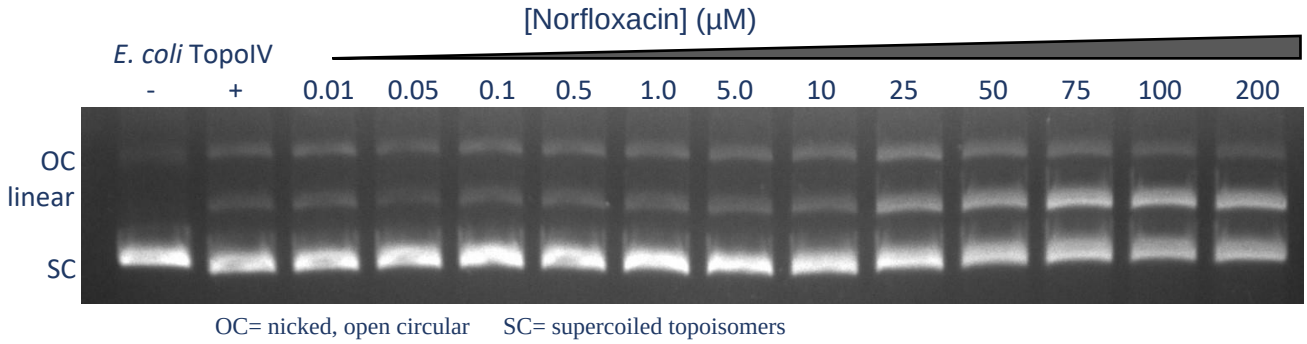
(supplied as 5X stock)

40 mM HEPES-KOH (pH 7.6)
100 mM Potassium Glutamate
10 mM Magnesium Acetate
10 mM dithiothreitol
50 ug/ml albumin

Cleavage Assay

1U of *E. coli* topo IV is incubated in 1X Assay Buffer with 0.5 µg of supercoiled pBR322 in the presence of norfloxacin in a reaction volume of 30 µl at 37°C for 30 minutes.
0.2% SDS and 0.1mg/ml Proteinase K are then added before a further incubation at 37°C for 30 minutes.
Gels can be run in the presence or absence of ethidium bromide, the former usually giving clearer results. Cleavage is detected by the presence of a linear band.

Gel run in the presence of 0.5 µg/mL ethidium bromide



OC= nicked, open circular SC= supercoiled topoisomers

Quality Control

Purity: The parC and parE subunits are purified to >95% purity as judged by SDS-polyacrylamide gel electrophoresis.

No activity was detectable when the single subunits were assayed alone

pBR322 was also incubated for 4 hrs in assay buffer + 10 mM $MgCl_2$ at 37 °C. These tests were negative for the formation of linear products, indicating the absence of nuclease contamination.

References

Peng, H. and Marians, K.J. (1999) Over-expression and purification of bacterial topoisomerase IV, in DNA Topoisomerase Protocols Vol. I (Bjornsti, M-A., and Osheroff, N. eds.), Humana Press, Totowa, N. Jersey p.163-169

Marians, K.J. and Hiasa, H. (1997) Mechanism of quinolone action. A drug-induced structural perturbation of the DNA precedes strand cleavage by topoisomerase IV. J. Biol. Chem., 272, 9401-9409.

Nurse, P., Bahng, S., Mossessova, E. and Marians, K.J. (2000) Mutational analysis of Escherichia coli topoisomerase IV. II. ATPase negative mutants of ParE induce hyper-DNA cleavage. J. Biol. Chem., 275, 4104-4111.