

E. coli Topoisomerase IV Decatenation Kit



Product Description (Product Numbers D4001, D4002, D4003 and D4004)

E. coli topoisomerase IV is prepared by overexpressing the parC 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 at a concentration of 10 U/μl in Dilution Buffer. Stable for 12 months undiluted. Store at -80°C. It is recommended that the enzyme is aliquoted to avoid repeated freeze-thaw cycles.

For *in vitro* laboratory research use only.

Dilution Buffer

40 mM HEPES-KOH (pH 7.6)
100 mM potassium glutamate
1 mM DTT
1 mM EDTA
40 % (v/v) glycerol

Assay Buffer (supplied as 5x stock)

50 mM HEPES-KOH (pH 7.6)
100 mM potassium glutamate
10 mM magnesium acetate
10 mM DTT
1 mM ATP
50 μg/ml albumin

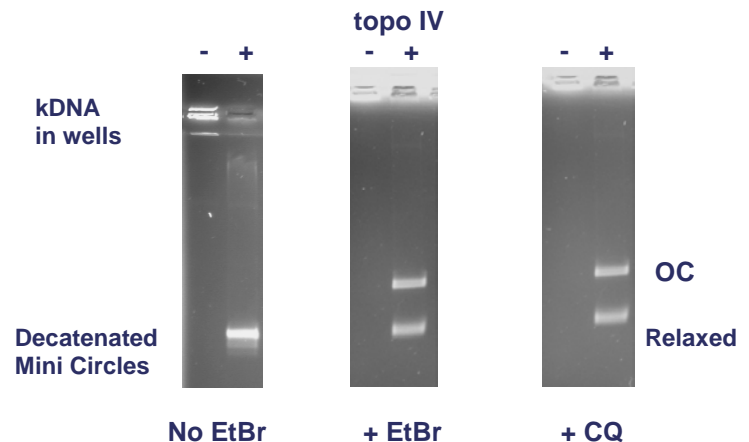
kDNA

Supplied at 100ng/μl
in 10mM Tris.HCl
(pH8.0), 1mM EDTA.

The kDNA should be
stored at 4°C.

Decatenation Assay

1 U of topoisomerase IV will decatenate 200 ng of kDNA when incubated in 1X assay buffer in a total reaction volume of 30 μl at 37°C for 30 minutes.
Gels can be run in the presence or absence of Ethidium Bromide or Chloroquine which will resolve nicked OC DNA from relaxed.



Quality Control

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

Reference

Peng, H. and Marians, K.J. (1999) Overexpression 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