Escherichia coli Topoisomerase IV Decatenation Assay Kits

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 minimum concentration of 10 U/µl in Dilution Buffer. However, we recommend that the enzyme is titrated into the assay to ascertain the minimum volume of enzyme required per assay to achieve full decatenation particularly if the kit is being used for drug screening purposes. Please refer to the protocol for more information: https://www.inspiralis.com/assets/TechnicalDocuments/E3.coli-topo-IV-Decatenation-Assay-Protocol.pdf
Store at -80°C.
It is recommended that for larger kits 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

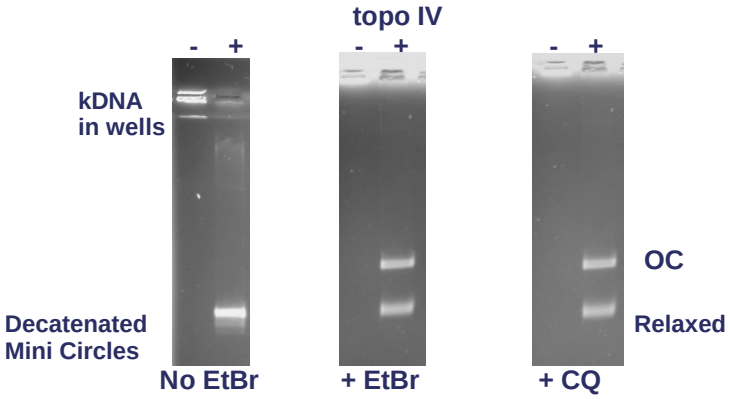
kDNA

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 (EtBr) or chloroquine (CQ) which will resolve nicked open circular (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