E. coli Topoisomerase IV

Product Description (Product Numbers T4001, T4002, T4003 and T4004)

E. coli Topoisomerase IV is prepared by overexpressing the parC and parE 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 5-10 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 supercoiling. 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/E2.coli-topo-IV-Relaxation-Assay-Protocol.pdf

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

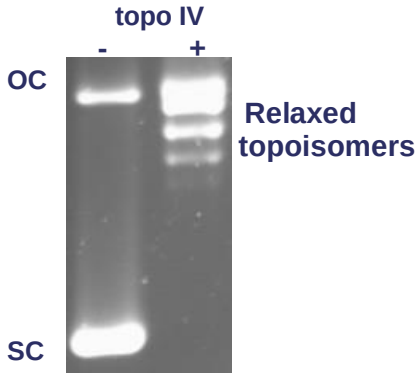
40 mM HEPES.KOH (pH 7.6)
100 mM potassium glutamate
1 mM DTT
1 mM EDTA
40 % (v/v) glycerol

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

Relaxation Assay

1 U of topoisomerase IV will relax 0.5 µg of supercoiled pBR322 when incubated in Assay Buffer in a total reaction volume of 30 µl at 37 °C for 30 minutes.
Gels are run in the absence of ethidium bromide or chloroquine.



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 pp.163-169