

E. coli Topoisomerase IV Relaxation Kit



Product Description (Product Numbers R4001, R4002, R4003 and R4004)

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 10 U/ μ l in Dilution Buffer.

Store at -80°C. (Stable for 12 months undiluted)

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)

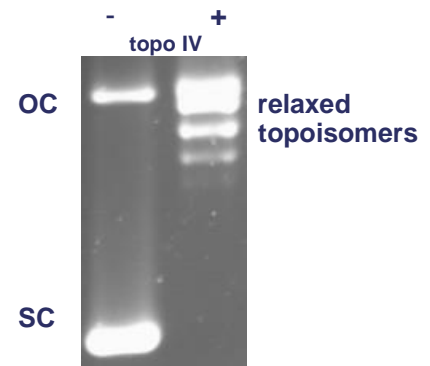
50 mM HEPES-KOH (pH 7.6)
100 mM potassium glutamate
10 mM magnesium acetate
10 mM DTT
1 mM ATP
50 μ g/ml albumin

DNA Storage Buffer

10 mM Tris.HCl (pH 7.5)
1 mM EDTA

Relaxation Assay

1 U of topoisomerase IV will relax 0.4 μ g of supercoiled pBR322 when incubated in Assay Buffer in a total reaction volume of 30 μ l 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