

E. coli Topo IV Assay and Dilution Buffers



Product Description (Product Numbers TR001, TD002 and TD003)

For use with *E. coli* topoisomerase IV enzyme and overexpressed cell extracts containing *E. coli* topoisomerase IV enzyme.

For *in vitro* laboratory research use only.

Dilution Buffer

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

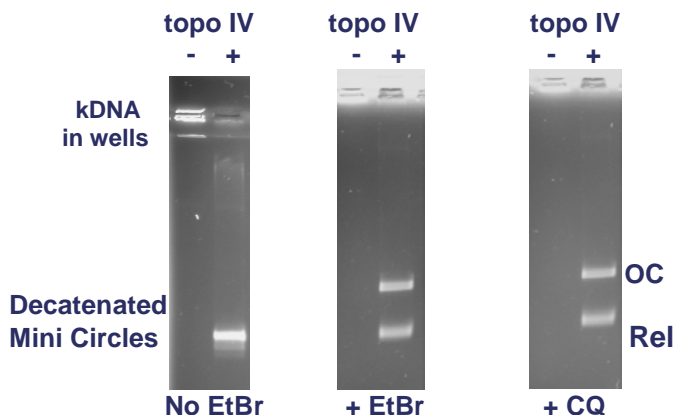
Assay Buffer (supplied as 5X stock)

40 mM HEPES.KOH (pH 7.6)
100 mM KCl
10 mM MgCl₂
10 mM DTT
1 mM ATP
50 µg/ml albumin

Decatenation Assay

1 U of topoisomerase IV will decatenate 200 ng of kDNA when incubated in 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.



Relaxation Assay

1 U of topoisomerase IV will relax 0.4 µg of supercoiled pBR322 when incubated in Assay Buffer in a total reaction volume of 30 µl at 37 °C for 30 minutes.

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