Pseudomonas aeruginosa Topoisomerase IV Decatenation Assay Kits

Product Description (Product Numbers PAD4001 and PAD4002)

P. aeruginosa topoisomerase IV is prepared by overexpressing the ParC and ParE subunits in *E. coli* and purified using in-house methods. It is supplied in Dilution Buffer as a heterotetramer complex at a minimum concentration of 10 U/µl (please see Certificate of Analysis for specific lot numbers). 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 https://www.inspiralis.com/assets/TechnicalDocuments/P-aeruginosa-Topo-IV-Decatenation-Assay-Protocol2.pdf. Kits are supplied with enzyme, assay and dilution buffers and kDNA substrate.
Store at -80 °C. For larger kits it is recommended that the enzyme is aliquoted to avoid repeated freeze-thaw cycles.
For *in vitro* laboratory research use only.

Dilution Buffer (1X)

50 mM Tris.HCl (pH 7.6)
50 mM sodium chloride
1 mM EDTA
2 mM DTT
50 % (w/v) glycerol

Assay Buffer (supplied as 5X stock)

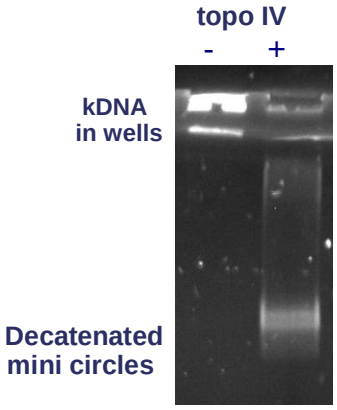
50 mM HEPES.KOH (pH 7.9)
6 mM magnesium acetate
4 mM DTT
1 mM ATP
100 mM potassium glutamate
2 mM spermidine
0.05 mg/ml albumin

kDNA Storage Buffer

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

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 are run in the absence of intercalators (e.g. ethidium bromide and chloroquine).



Quality Control

1) Purity: *P. aeruginosa* topo IV is purified to > 95 % purity as judged by SDS-polyacrylamide gel electrophoresis.
2) No activity was detectable when the single subunits were assayed alone.