S. aureus Topoisomerase IV Decatenation Kit

Product Description (Product Numbers SAD4001 and SAD4002)

S. aureus topoisomerase IV is purified after heterologous expression in *E. coli* and is supplied as a complex of the subunits.
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/S2.aureus-Topo-IV-Decatenation-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 (1X)

50 mM Tris.HCl (pH 7.5)
1 mM DTT
1 mM EDTA
40 % (w/v) glycerol

Assay Buffer (supplied as 5X stock)

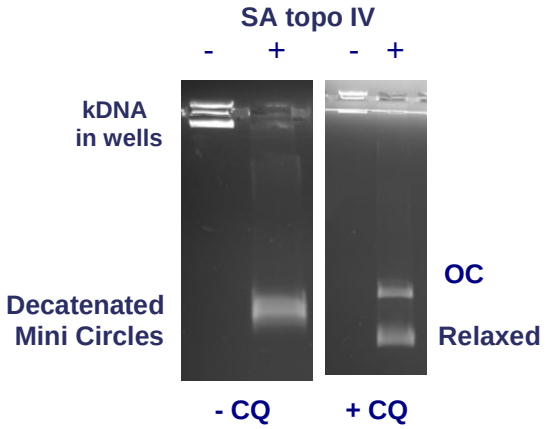
50 mM Tris.HCl (7.5)
5 mM $MgCl_2$
5 mM DTT
1.5 mM ATP
350 mM potassium glutamate
0.05 mg/ml albumin

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 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

1) Purity: *S. aureus* topo IV is purified to > 95 % purity as judged by SDS-polyacrylamide gel electrophoresis. 2) pBR322 was also incubated for 4 hrs in assay buffer + 10 mM $MgCl_2$ at 37 °C. These tests were negative for the formation of linear products, indicating the absence of nuclease contamination. (3) No activity was detectable when the single subunits were assayed alone.