

S. aureus Topo IV Cleavage Assay Kit



Product Description (Product Number SATC001)

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 concentration of 2.4 μM in Dilution Buffer. Cleavage activity is 2.5U/ μl .

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)

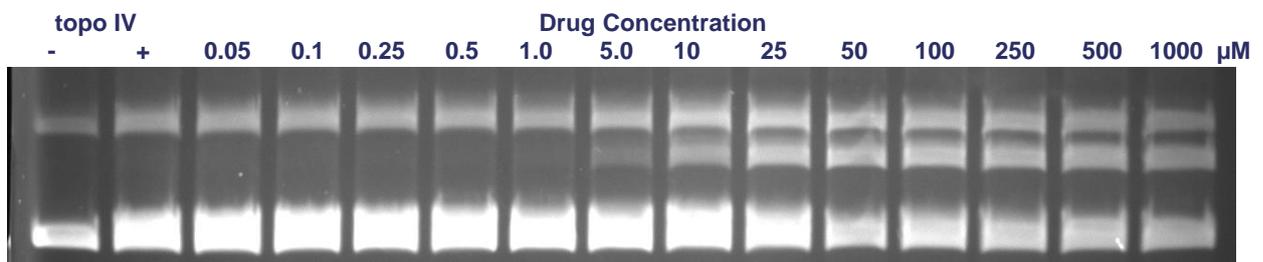
50 mM Tris.HCl (7.5)
5 mM MgCl_2
5 mM DTT
350 mM potassium glutamate
0.05 mg/ml albumin

Cleavage Assay

1U *S. aureus* Topo IV (0.1 μl) is incubated with 0.5 μg of supercoiled pBR322, in the presence of norfloxacin, in a reaction volume of 30 μl at 37°C for 1 hour in Assay Buffer.

0.2% SDS and 0.1mg/ml Proteinase K are added before a further incubation at 37°C for 30 minutes. Cleavage is detected by the presence of a linear band.

Titration of Norfloxacin under cleavage conditions with *S.aureus* Topo IV



Quality Control

Purity: *S. aureus* topoisomerase IV is purified to > 95 % purity as judged by SDS-polyacrylamide gel electrophoresis.

No activity was detectable when the single subunits were assayed alone

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.