

# *A.baumannii* Topoisomerase IV Decatenation Kit



## Product Description (Product Numbers ABD001 and ABD002)

*A.baumannii* topoisomerase IV is prepared by overexpressing the parC and par E subunits in *E. coli* and purifying them by methods adapted from Peng and Mariani, 1999. It is supplied as a heterotetramer complex. The enzyme is supplied at a minimum concentration of 10 U/ $\mu$ l in Dilution Buffer. Store at  $-80^{\circ}\text{C}$ . It is recommended that the enzyme is aliquoted to avoid repeated freeze-thaw cycles.

**For *in vitro* laboratory research use only.**

### Dilution Buffer

100 mM potassium phosphate (pH 7.6)  
1 mM DTT  
1 mM EDTA  
30 % (v/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  
50  $\mu$ g/ml albumin

### kDNA

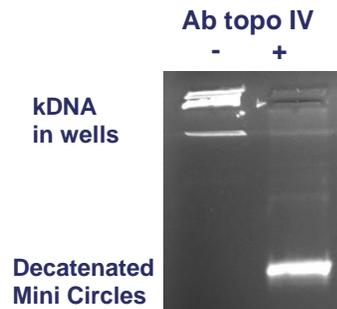
Supplied at 100ng/ $\mu$ l  
in 10mM Tris.HCl  
(pH8.0), 1mM EDTA.

The kDNA should be  
stored at  $4^{\circ}\text{C}$ .

### 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  $\mu$ l at  $37^{\circ}\text{C}$  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.



### Quality Control

Purity: The ParC and ParE subunits are purified to  $>95\%$  purity as judged by SDS-polyacrylamide gel electrophoresis.

### Reference

Peng, H. and Mariani, 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 p.163-169