M. tuberculosis Gyrase (HIS) Decatenation kit

Product Description (Product Numbers MTD001 and MTD002.)

M. tuberculosis gyrase is a C-terminal HIS-tagged protein purified after heterologous expression in *E. coli* and is supplied as an A_2B_2 complex. The enzyme is supplied at a minimum activity of 2 U/µl in Dilution Buffer. 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 7.9)
5 mM DTT
30 % (w/v) glycerol

Assay Buffer (supplied as 5X stock)

40 mM Tris.HCl (7.5)
10 mM NaCl
10 mM DTT
1 mM ATP
250 mM potassium glutamate
6 mM Magnesium Acetate
0.5 mg/ml albumin

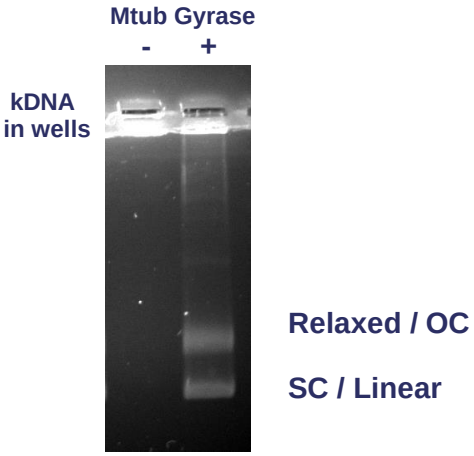
kDNA

Supplied at 100ng/µl in 10mM Tris.HCl (pH8.0), 1mM EDTA.

The kDNA should be stored at 4°C.

Decatenation Assay

1 U of M. tuberculosis gyrase 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 can be run in the presence or absence of Ethidium Bromide or Chloroquine which will resolve nicked OC DNA from relaxed. Mtub gyrase decatenation results in a range of DNA forms which migrate to form 2 distinct bands of relaxed and supercoiled DNA.



Quality Control

1) Purity: *M. tuberculosis* DNA gyrase is purified to > 95 % purity as judged by SDS-polyacrylamide gel electrophoresis.

2) No activity was detectable when the single subunits were assayed alone.

3) kDNA is purified to give a ratio of OD260 to OD280 ratio of 1.8 -1.9 with no detectable free mini-circles when catenated kDNA is run on a 1 % agarose gel. Each batch is tested for decatenation with purified topoisomerase IV.