

M. tuberculosis Gyrase Supercoiling Assay Kit



Product Description (MTS001 and MTS002)

M. tuberculosis gyrase is a C-terminal HIS-tagged protein purified after heterologous expression in *E. coli* and is supplied as an A₂B₂ complex. The enzyme is supplied at a concentration 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

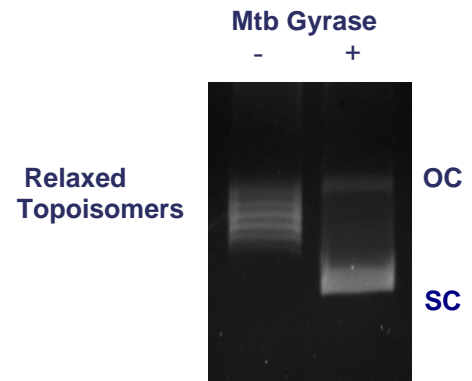
Assay Buffer (supplied as 5X stock)

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

Supercoiling Assay

1 U of gyrase is incubated with 0.5 μg of relaxed pBR322 in a reaction volume of 30 μl at 37°C for 30 minutes in Assay Buffer.

Gels are run in the absence of Ethidium Bromide or Chloroquine.



Quality Control

- 1) Purity: *M. tuberculosis* gyrase 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 MgCl₂ 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