M. tuberculosis Gyrase Supercoiling Assay Kit

Product Description (MTS001 and MTS002)

M. tuberculosis DNA 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 concentration of 5 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 supercoiling. 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/M.tuberculosis-Gyrase-Supercoiling-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 7.9)
5 mM DTT
30 % (w/v) glycerol

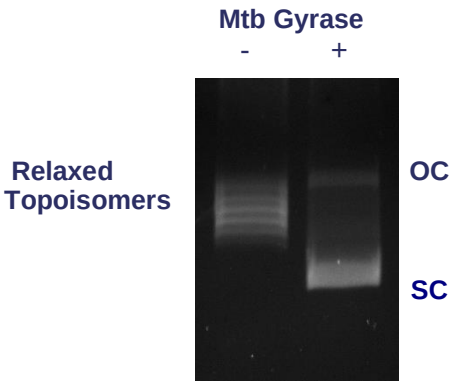
Assay Buffer (supplied as 5X stock)

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 DNA 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 $MgCl_2$ 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