M.tuberculosis Gyrase Cleavage Assay Kit

Product Description

(Product Numbers MTGC001, MTGC002)

M. tuberculosis DNA 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 3.5 µM in Dilution Buffer and is suitable for cleavage assays. Cleavage activity is a minimum of 2.5 – 5.0 U/µl. Maximum cleavage can be obtained with 0.2 µl in the presence of 100 µM Moxifloxacin in a 30 µl reaction (see typical titration below).

Store at -80°C. **For *in vitro* laboratory research use only.**

Dilution Buffer

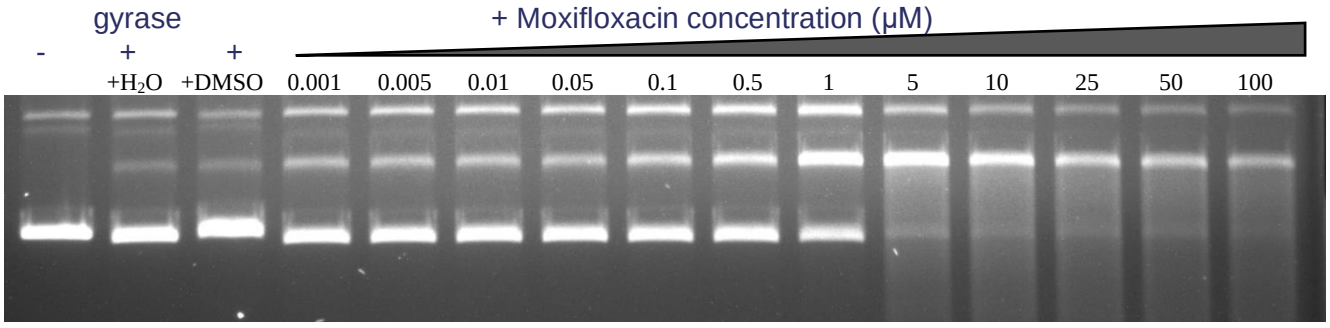
50 mM Tris.HCl (pH 7.9)
5 mM DTT
30 % (w/v) glycerol

Assay Buffer (supplied as 5X stock)

50 mM HEPES. KOH (pH 7.9)
6 mM magnesium acetate
4 mM DTT
100 mM potassium glutamate
2 mM spermidine
0.05 mg/ml albumin

Cleavage Assay

Gyrase is incubated with 0.5 µg of supercoiled pBR322 in a reaction volume of 30 µl at 37 °C for 1 hour in Assay Buffer in the presence of Moxifloxacin. 0.2 % SDS and 0.1 mg/ml Proteinase K are added before a further incubation at 37 °C for 30 minutes.



Quality Control

Purity: The A and B subunits are purified to > 95 % purity as judged by SDS-polyacrylamide gel electrophoresis.

Endonuclease assay: 0.5 µg relaxed pBR322 incubated with 0.5 U of DNA gyrase for 1 hour at 37 °C in the presence of 1 mM ATP shows no detectable conversion of supercoiled DNA to either open circular or linear forms when assayed by agarose gel electrophoresis.