S. aureus DNA Gyrase for ATPase Assays

Product Description (Product Number #ATPSAG001)

S.aureus DNA gyrase was prepared after heterologous expression in *E. coli* and is supplied as an A_2B_2 complex at a concentration of 1U/10µl (0.5 µM) in Dilution Buffer.
Store at -80 °C.
For *in vitro* laboratory research use only.

Assay Buffer (supplied as 5X stock)

40 mM HEPES. KOH (pH 7.6)
10 mM magnesium acetate
10 mM DTT
500 mM potassium glutamate
0.05 mg/ml albumin

Dilution Buffer

50 mM Tris.HCl (pH 7.5)
1 mM DTT
1 mM EDTA
40 % (w/v) glycerol

DNA Substrate

0.33 µM pBR322 linearised with EcoR1.
Use at 3 µl/100 µl assay to give a final concentration of 10 nM.

ATP

30mM

ATPase Assay

The coupled enzyme ATPase assay is based on the conversion of phosphoenolpyruvate (PEP) to pyruvate kinase (PK) coupled to the conversion of pyruvate to lactate by lactate dehydrogenase (LDH). This step requires NADH which is oxidized to NAD+. NADH absorbs strongly at 340 nM but NAD+ does not, enabling the reduction of NADH over time to be followed by monitoring the decrease in absorbance at 340 nM.

Assays should be carried out under the following conditions at 25°C in clear, 96-well flat bottomed plates.

1 U of DNA gyrase is incubated at 25 °C in a final volume of 100 µl containing 1X assay buffer, 800 µM phosphoenolpyruvate, 400 µM NADH, 1.5 µl phosphokinase/lactate dehydrogenase (PK/LDH) enzyme mix (Sigma P0294) plus or minus DNA and inhibitors. The mix is equilibrated for 10 mins at 25 °C. Reactions are then initiated by the addition of ATP (Mg^{2+}) to 2 mM and the decrease in A_{340} measured over time.