E. coli Gyrase for ATPase Assays

Product Description (Product Number ATPG001)

Gyrase subunits were prepared from the over producing strains JMtacA and JMtacB (Hallett, *et al*., 1990) and the Gyr B subunit then further purified. It is supplied as an A_2B_2 complex at a concentration of 0.5 μM in Dilution Buffer.

Store at -80 °C.

For *in vitro* laboratory research use only.

(see https://www.inspiralis.com/technical/protocols/gyrase/)

Assay Buffer (supplied as 5X stock)

50 mM Tris.HCl (pH 7.5)
1 mM EDTA
5 mM $MgCl_2$
5 mM DTT
10 % (w/v) glycerol

Dilution Buffer

50 mM Tris.HCl (pH 7.5)
100 mM KCl
2 mM DTT
1 mM EDTA
50 % (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	30 mM
PEP	80 mM
PK/LDH	
NADH	20 mM

ATPase Assay

The coupled enzyme ATPase assay links the hydrolysis of ATP to the conversion of NADH to NAD^+. Phosphoenolpyruvate (PEP) is converted to pyruvate kinase (PK) with the conversion of ADP to ATP. The pyruvate is converted to lactate by lactate dehydrogenase (LDH) oxidising NADH 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. It is assumed that the oxidation of each molecule of NADH is linked to the hydrolysis of one molecule of ATP. The activity is stimulated by binding DNA.

Assays should be carried out under the following conditions at 37°C in clear, 96-well flat bottomed plates

1 U (10 μL of 500nM) of gyrase is incubated at 37 °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 37 °C. Reactions are then initiated by the addition of ATP (Mg^{2+}) to 2 mM and the decrease in A_{340} measured over time.

Reference

Hallett, P., Grimshaw, A.J., Wigley, D.B. and Maxwell, A. (1990) Cloning of the DNA gyrase genes under *tac* promoter control: overproduction of the gyrase A and B proteins. *Gene* 93: 139-142