Gyrase B 43 kDa Domain

(isolated from *Escherichia coli*)

Product Description (Product Numbers B43001, B43005, B43100)

Gyrase B 43 kDa domain is supplied at a concentration of 2.6 mg/ml in Dilution Buffer. The protein has been purified by affinity and ion exchange chromatography and is guaranteed >95% pure as assessed by SDS-PAGE. The enzyme is free of contaminating non-specific ATPases as shown by novobiocin inhibition under the conditions below. Store at -80°C.

For *in vitro* laboratory research use only.

Dilution Buffer

50 mM Tris.HCl (pH 7.5)
100 mM KCl
2 mM DTT
1 mM EDTA
10 % (w/v) glycerol

3X Assay Buffer

(adjusted to allow for the buffer constituents in the B43 domain)
100 mM Tris.HCl (pH 7.5)
15 mM $MgCl_2$
10 mM DTT
2.1 mM EDTA
20 % (w/v) glycerol

ATPase Assay

The coupled-enzyme ATPase assay is based on the conversion of phosphoenolpyruvate (PEP) to pyruvate by pyruvate kinase (PK) coupled to the conversion of pyruvate to lactate by lactate dehydrogenase (LDH). This last 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:

The N-terminal Gyr B43 domain is incubated in 50 mM Tris HCl (pH 7.5), 1 mM EDTA, 10 % (w/v) glycerol, 25 mM KCl, 4 mM dithiothreitol (DTT), 5 mM $MgCl_2$, 800 µM PEP, 400 µM NADH, 1 % (v/v) PK/LDH (pyruvate kinase-lactate dehydrogenase mixture in 50% (w/v) glycerol,100 mM KCl, 10 mM HEPES (pH 7.0)) and 2 mM ATP.
The 43 kDa protein is added to the reaction at a final concentration of 20 µM and allowed to incubate with the compounds in the microtitre plate for 10 minutes before the addition of the ATP.

Reactions are initiated by the addition of ATP at a final concentration of 2 mM.
The decrease in A_{340} is measured for 20 minutes.

A typical 150 ul reaction will have the following:
50 µl Gyrase B43 at a final concentration of 20 µM, 50 µl 3X ATPase buffer, 3 µl 20 mM NADH (not supplied), 1.5 µl 80 mM PEP (not supplied) and 1.5 µl PK/LDH mix (not supplied). The volume is made up to 150 µl with ultra pure water and the ATP. For drug inhibition, pre-incubate the reaction mix with the drug for 10 minutes prior to the addition of the ATP.

Reference

Ali, J.A., Jackson, A.P., Howells, A.J. and Maxwell, A. (1993). The 43 kDa N-terminal fragment of the gyrase B protein hydrolyses ATP and binds coumarin drugs. *Biochemistry* **32**, 2717-2724