E. coli Topoisomerase IV for ATPase Assays

Product Description (Product Number ATPT001)

Topoisomerase IV is prepared by overexpressing the parC and parE subunits in *E. coli* and purifying them by methods adapted from Peng and Marians, 1999. It is supplied as a heterotetramer complex. The enzyme is supplied at a concentration of 1 U/10 µl 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)
100 mM KGlutamate
10 mM MgAcetate
1 mM DTT
50 µg/ml albumin

Dilution Buffer

40 mM HEPES.KOH (pH 7.6)
100 mM KGlutamate
1 mM DTT
1 mM EDTA
40 % (v/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 37°C in clear, 96-well flat bottomed plates

1 U of topoIV 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.