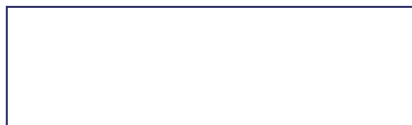


## *E. coli* Topoisomerase IV for ATPase Assays



### 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 Mariani, 1999. It is supplied as a heterotetramer complex. The enzyme is supplied at a concentration of 1 U/10  $\mu$ l 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  $\mu$ g/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  $\mu$ M pBR322 linearised with EcoR1.  
Use at 3  $\mu$ l/100  $\mu$ l 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<sup>+</sup>. NADH absorbs strongly at 340 nM but NAD<sup>+</sup> 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  $\mu$ l containing 1X assay buffer, 800  $\mu$ M phosphoenolpyruvate, 400  $\mu$ M NADH, 1.5  $\mu$ l 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<sup>2+</sup>) to 2 mM and the decrease in A<sub>340</sub> measured over time.