

Escherichia coli Topoisomerase IV Cleavage Assay

Introduction

Topo IV is a type II topoisomerase that can relax supercoiled DNA at the expense of ATP hydrolysis. It is present in most bacteria but absent from higher eukaryotes. The enzyme acts by creating a double-strand break in one segment of DNA (forming a 'cleavage-complex'), passing another DNA segment through it and resealing the break. Some compounds such as the quinolones inhibit the enzyme by stabilising the cleavage complex. In this assay, the substrate, supercoiled pBR322, is incubated under relaxation conditions. It is then treated with SDS and proteinase K to trap any cleavage complexes and the products can then be analysed by agarose gel electrophoresis.

Materials

***E. coli* Topo IV Assay Buffer** : 40 mM HEPES.KOH (pH 7.6), 100 mM potassium glutamate, 10 mM magnesium acetate, 10 mM DTT, 1 mM ATP, and 50 µg/mL albumin (supplied as 5X). Store at -20°C or below.

Dilution Buffer : 40 mM HEPES.KOH (pH 7.6), 100 mM potassium glutamate, 1 mM DTT, 1 mM EDTA, and 40% (v/v) glycerol. (supplied as 1X). Store at -20°C or below.

Enzyme : *E. coli* Topo IV (see data sheet for concentration). Store at -80°C. (NOTE 1)

Plasmid : Supercoiled pBR322 (supplied at 1 µg/µL). Store at -20°C or below.

GSTEB: 40% (w/v) Glycerol, 100 mM Tris-HCl pH8, 1 mM EDTA, 0.5 mg/mL Bromophenol Blue. (Supplied at 10X)

Other materials not included in the kit

SDS: 2% (w/v) sodium dodecyl sulphate in water

Proteinase K: 10 mg/mL in water

Method

On ice, set up a MIX of (per assay): Assay buffer (6 µL of 5X buffer) (NOTE 2), supercoiled pBR322 (0.5 µL) and water (17.5 µL). 24 µL of MIX are needed per assay.

Set up the appropriate number of 1.5 mL tubes and aliquot 24 µL of MIX into each tube.

If the compounds are dissolved in DMSO then add 3 µL of DMSO to tubes 1 and 2 (but see NOTE 3).

Add 3 µL of the test compounds to the other tubes as appropriate.

Mix briefly.

Add 3 µL of dilution buffer to tube 1 (NOTE 4).

Dilute the enzyme in dilution buffer then add 3 µL of this to the remaining tubes. (see NOTE 1)

Mix by gentle vortexing and incubate for 60 minutes at 37°C.

After incubation add 3 µL of 2% (w/v) SDS and 1.5 µL of 10 mg/mL Proteinase K.

Mix briefly by vortexing and incubate for 30 minutes at 37°C.

Stop reaction by adding 30 µL of 2X GSTEB and 30 µL of chloroform/isoamyl alcohol (24:1, v:v). (NOTE 4)

Vortex vigorously ~5 secs and centrifuge for 2 minutes.

bands. Longer runs will give greater separation. The gels shown were run in the absence of intercalators but the inclusion of an intercalator (e.g. 5 $\mu\text{g}/\text{mL}$ chloroquine may help to resolve any topoisomers away from the linear band.

- 7) Once the reactions have been stopped with the GSTEB and mixed, it is possible to store them overnight at 4°C although it is preferable to load them on a gel as soon as possible.