Human Topoisomerase II Cell Extract Assay Kit

Introduction

Human topo II is able to decatenate interlinked double-stranded DNA molecules. In this assay, the substrate is kinetoplast DNA (kDNA) from *Crithidia fasciculata* which consists of a network of many minicircles (2.3 Kb) and a few maxicircles. The enzyme will release the minicircles from this network. The substrate will remain in the wells after gel electrophoresis (although it is not always visible) while the released minicircles will migrate into the gel where they can be seen after staining with ethidium bromide. This kit can be used to identify the presence of topo II activity in a cell extract.

Materials

Human Topo II assay buffer : 50 mM Tris.HCl (pH 7.5), 125 mM NaCl, 10 mM $MgCl_2$, 5 mM DTT, and 100 µg/mL albumin (supplied at 10X). Store at -20°C or below.

Dilution Buffer : 50 mM Tris.HCl (pH 7.5), 100 mM NaCl, 1 mM DTT, 0.5 mM EDTA, 50% (v/v) glycerol, and 50 µg/mL albumin (supplied as 1X). Store at -20°C or below.

ATP: 30 mM ATP (supplied as 30X). Store at -20°C or below.

Substrate : kDNA (supplied at 100 ng/µL). Store at 4°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

Enzyme : Human Topo II

Method (example assay set up)

On ice, set up a MIX of Assay Buffer (3 µL of 10x buffer per assay) (NOTE 2), ATP (1 µL), kDNA (2 µL per assay) and water.

27 µL of MIX are required per assay.

Set up the appropriate number of 1.5 mL tubes and aliquot 27 µL of MIX into each tube.

Add 3 µL of dilution buffer (NOTE 2) to tube 1.

Add 3 µL of the sample extract to the sample tube (see NOTE 1).

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

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

Vortex briefly ~5 secs and centrifuge for 2 minutes.

Load 20 µL of aqueous (upper blue) phase onto a 1% (w/v) agarose gel. (NOTE 3)

Run at 85 V for approximately 1 hour.

Stain with ethidium bromide (1 µg/mL in water) (15 mins), destain (5-10 mins) in water (NOTE 4) and visualise with a transilluminator or gel documentation system.

Notes

1) The final concentration of assay buffer should be 1X.

2) The dilution buffer contains a high concentration of glycerol therefore the total dilution buffer added should not exceed 10% of the final volume.

3) The agarose gels shown in the results are run in Tris-acetate-EDTA buffer (40 mM Tris-acetate, 1 mM EDTA) but TPE (36 mM Tris, 30 mM sodium phosphate, 1 mM EDTA pH 7.8) or TBE (89 mM Tris, 89 mM borate, 2 mM EDTA pH 7.6) buffers can also be used. Gels should be run at about 4-5 V/cm. Gels are usually run until the bromophenol blue dye has run at least 4-5 cm. This will be enough to see the decatenated mini-circles.

4) Gels can be run in the presence or absence of ethidium bromide. In the absence of ethidium, the decatenated mini-circles will usually run as a single band (or as a number of topoisomers) while in the presence of the stain, two bands are frequently present consisting of supercoiled and open-circular (relaxed) mini-circles respectively. This separation can also be achieved by including other intercalators such as chloroquine in the gel. The gels below show the typical appearance of gels run in the presence or absence of an intercalator (chloroquine)