Wheat Germ Topo I Relaxation Assay

Introduction

Wheatgerm topo I is similar to eukaryoytic type I topoisomerases and is able to relax supercoiled DNA. In this assay, the substrate is supercoiled pBR322 which is relaxed by the enzyme. The two forms of the plasmid can be separated by agarose gel electrophoresis. It can be used to determine the activity of compounds as inhibitors of topo I.

Materials

Wheat Germ Topo I assay buffer : 50 mM Tris.HCl (pH 7.9), 1 mM EDTA, 1 mM DTT, 20% (v/v) glycerol, and 50 mM NaCl. (Supplied as 2X)

Dilution Buffer : 50 mM Tris.HCl (pH 7.9), 500 mM NaCl, 1 mM DTT, 1 mM EDTA, and 50% (w/v) glycerol. (Supplied as 1X)

Enzyme : Wheat Germ Topo I (Supplied at a minimum 5 U/μL) (NOTE 1).

Plasmid : Supercoiled pBR322 (Supplied at 1 μg/μL).

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

Method

On ice, Set up a MIX of assay buffer (15 μL of 2X buffer per assay) (NOTE 2), supercoiled pBR322 (0.5 μL per assay) and water (18.5 μL per assay). 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 to tube 1 (NOTE 3).

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

Mix briefly (gentle vortexing or pipetting) 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 ~2 secs and centrifuge for 2 minutes.

Load 20 μL of aqueous phase onto a 1 % (w/v) agarose gel (+/- chloroquine as appropriate).

Run at 80V for approximately 2 hours (NOTE 4)

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

Example Results

Determination of enzyme activity

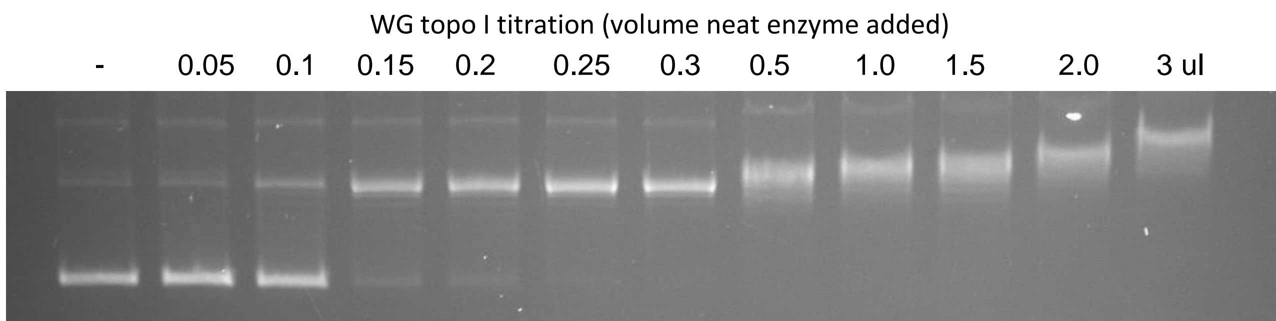
The gel below shows the results of a gel-based relaxation to determine the amount of enzyme to use in a subsequent relaxation or unwinding assay. This is performed with supercoiled pBR322. And set up as follows:

A mix was prepared for 12 reactions with 180 µL Assay Buffer (2X), 7 µL supercoiled pBR322 and 161 µL water.

27 µL of this mix were added to each tube.

Serial dilutions of the enzyme were made in dilution buffer (DB) then 3.0 µL added to the reactions.

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14
MIX (µL)	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
DB (µL)	3													
Enzyme (µL)		3	3	3	3	3	3	3	3	3	3	3	3	



In this case approximately 0.25 µL of the neat enzyme is required to give full relaxation. It is not recommended to store the diluted enzyme as it can lose activity.

Notes

1) Enzyme is supplied as at a minimum of 2 U/µL (1 U is the amount of topo I required to just relax 0.5 µg of supercoiled pBR322) but it can be significantly more. Thus if you require a known amount of relaxation (e.g. 90% relaxation) then an enzyme dilution series, where the enzyme is titrated into the assay, is recommended.

2) Final concentration of assay buffer should be 1X.

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

4) The agarose gel shown in the results was run in Tris-acetate-EDTA buffer.