May 22, 2018

Assay of topoisomerase I activity

PLOS One

DOI
https://dx.doi.org/10.17504/protocols.io.p99dr96

Yuan Yao[1]

[1]Inner Mongolia People`s Hospital

Yuan Yao

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.p99dr96

External link: https://doi.org/10.1371/journal.pone.0200688

Protocol Citation: Yuan Yao 2018. Assay of topoisomerase I activity. **protocols.io** https://dx.doi.org/10.17504/protocols.io.p99dr96

Manuscript citation:
Yao Y, Ma Y, Chen X, Bade R, Lv C, Zhu R (2018) Absence of RstA results in delayed initiation of DNA replication in *Escherichia coli*. PLoS ONE 13(7): e0200688. doi: 10.1371/journal.pone.0200688

Protocol status: Working
We use this protocol and it's working

Created: May 22, 2018

Last Modified: May 22, 2018

Protocol Integer ID: 12321

Keywords: Assay of Topoisomerase I activity, assay of topoisomerase, topoisomerase, assay

Attachments

PDF

Topoisomerase Assays...

Materials

MATERIALS

- Topoisomerase I (E.coli) - 500 units **New England Biolabs Catalog #**M0301L
- pUC18 **addgene Catalog #**50004
- 6X Blue Loading Dye **Gold Biotechnology Catalog #**L002

Add 2 µl of 10×topoisomerase I reaction buffer and 400 ng pUC19 plasmid DNA (Takara, Japan) to each of a series of 1.5-ml microcentrifuge tubes on ice;Adjust volumes with distilled water so that the final reaction volume in each tube, including that of the protein or extract added in step 2, is 20 µl.

1

Add various amounts of purified RstA or one unit E. coli topoisomerase I protein (NEB, USA) to the tubes, then incubate 10 min at 37°C.

2

Add 4 µl of 6×loading dye to each tube and load contents on an 0.8% agarose gel. Run gel 2h at 5 to 10 V/cm.

3

Stain gel with ethidium bromide, destain briefly with water;

4

Photograph the gel illuminated with a UV transilluminator.

5