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Assay of topoisomerase I activity

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Protocol status: Working

We use this protocol and it's working

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Attachments



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 μ l of 10 $\times$ 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 μ l.

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 μ l of 6 $\times$ 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