
Topoisomerase II α Assay Kit (Cat No. TA-001)

I. Reagents*

- A. 10x Reaction Buffer (250 μ l): 0.5 M Tris-HCl, pH 8.0, 10 mM DTT, 0.1 M MgCl₂, 20 mM ATP, 1 M NaCl
- B. BSA (250 μ l, 2 mg/ml)
- C. Kinetoplast DNA (25 μ g, 0.25 mg/ml)
- D. Topoisomerase II alpha (100 units, 1 unit/ μ l)
- E. 6% SDS (250 μ l)
- F. 3 mg/ml proteinase K (250 μ l)
- G. 5x Loading Buffer (0.5 ml)
- H. 10x Control Buffer (250 μ l): 0.5 M Tris-HCl, pH 8.0, 10 mM DTT, 0.1 M MgCl₂, 1 M NaCl

**All reagents should be completely thawed, mixed by vortex, and briefly centrifuged. Except reagent E, which needs to be kept at room temperature, all reagents should be kept on ice during the preparation of reaction mixture described below and returned to below -70°C immediately after use.*

II. *In vitro* decatenation assay

Step 1. Prepare master mix for N number of reactions (10 μ l/reaction)^{a, b}:

Reagent A	1x (N+1) μ l
Reagent B	1x (N+1) μ l
Reagent C	1x (N+1) μ l

- Step 2.** Adjust final volume of master mixture to 6x (N+1) μ l by adding deionized dH₂O^b.
- Step 3.** Transfer aliquot (6 μ l) to reaction tubes.
- Step 4.** Add chemical or protein factor of your research interest.
- Step 5.** Add 1 unit of topoisomerase II α (**Reagent D**) or nuclear extract^c.
- Step 6.** Adjust the final reaction volume to 10 μ l by adding deionized dH₂O^b.
- Step 7.** Incubate at 37 $^{\circ}$ C for 15 min.
- Step 8.** Add 1 μ l aliquot of **Reagents E & F**.
- Step 9.** Incubate for an additional 30 min at 37 $^{\circ}$ C.
- Step 10.** Add 3 μ l of **Reagent G**, and resolve the entire mixture on an agarose gel (1 %).
- Step 12.** Following electrophoresis, stain the gel in ethidium bromide (0.5 μ g/ml) for 45 min.
- Step 13.** Incubate the gel in MgSO₄ (1 mM) for 1 hr.
- Step 14.** Photographing and densitometric analysis of visualized DNA topoisomers.

- a. For reaction in higher volume, proportionally increase the amount of reagents described in this manual.
- b. For control reaction lacking ATP, use **Reagent H** in place of **Reagent A**.
- c. Not provided.