

Human Topoisomerase II Relaxation Assay Kit



Product Description (Product Numbers HTR201, HTR202, HTR203 and HTR204)

Human topoisomerase II is prepared by overexpressing the enzyme in baculovirus-infected insect cells (*Spodoptera frugiperda*) and purifying it by methods developed in-house. The enzyme is supplied at a concentration of 10 U/ μ l in Dilution Buffer with supercoiled DNA substrate.

Store at -80 °C. (Stable for 3 months undiluted.) It is recommended that the enzyme is aliquoted to avoid repeated freeze-thaw cycles.

For *in vitro* laboratory research use only.

Dilution Buffer

50 mM Tris.HCl (pH 7.5)
100 mM NaCl
1 mM DTT
0.5 mM EDTA
50 % (v/v) glycerol
50 μ g/ml albumin

Assay Buffer (supplied as 10X stock)

50 mM Tris.HCl (pH7.5)
125 mM NaCl
10 mM MgCl₂
5 mM DTT
100 μ g/ml albumin

ATP (30X stock)

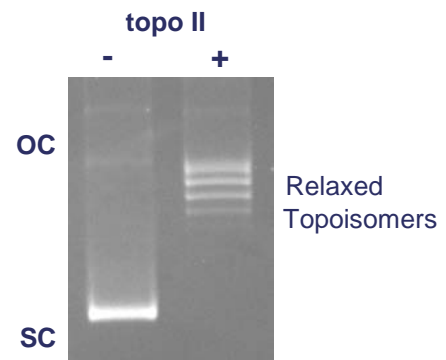
30 mM ATP

Relaxation Assay

A typical reaction will contain 3 μ l of (10x) Assay Buffer, 1 μ l of (30x) ATP, 0.5 μ l of supercoiled pBR322 (1 μ g/ μ l), plus human topo II, in a total volume of 30 μ l.

1 U of human topo II will relax 0.5 μ g of supercoiled pBR322 when incubated in 1X Assay Buffer plus 1 mM ATP in a total reaction volume of 30 μ l at 37 °C for 30 minutes.

Gels should be run in the absence of ethidium bromide or chloroquine (CQ).



Quality Control

1) Purity: Human topoisomerase II is purified to > 95 % purity as judged by SDS-polyacrylamide gel electrophoresis. 2) Tests for human topoisomerase I contamination by looking for relaxation of sc pBR322 under topoisomerase I assay conditions were negative. 3) kDNA or pBR322 were also incubated for 4 hrs in assay buffer (+ 10 mM MgCl₂) at 37 °C. These tests were negative for the formation of linear products, showing the absence of nuclease contamination.