

# Human Topoisomerase I Assay Kit



## Product Description (Product Numbers HTR101, HTR102, HTR103 and HTR104)

Human topoisomerase I is prepared by overexpressing in baculovirus-infected insect cells (*Spodoptera frugiperda*) and purifying it by methods adapted from Stewart *et al.*, 1996. The enzyme is supplied at a concentration of 10 U/ $\mu$ l in Dilution Buffer with sufficient supercoiled DNA substrate for the supplied number of units.

Store at  $-80^{\circ}\text{C}$ . (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

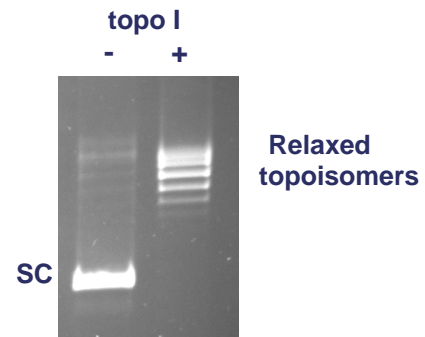
10 mM Tris.HCl (pH 7.5)  
1 mM DTT  
1 mM EDTA  
50 % (v/v) glycerol  
100  $\mu$ g/ml albumin

### Assay Buffer (supplied as 10X stock)

20 mM Tris.HCl (pH7.5)  
200 mM NaCl  
0.25 mM EDTA  
5 % glycerol

### Relaxation Assay

1 U of topoisomerase I will relax 0.5  $\mu$ g of supercoiled pBR322 when incubated in Assay Buffer in a total reaction volume of 30  $\mu$ l at  $37^{\circ}\text{C}$  for 30 minutes. Gels are run in the absence of ethidium bromide or chloroquine.



### Quality Control

1) Purity: Human topoisomerase I is purified to  $> 95\%$  purity as judged by SDS-polyacrylamide gel electrophoresis. 2) Tests for human topoisomerase II contamination by looking for decatenation of kDNA under topoisomerase II assay conditions were negative. 3) kDNA or pBR322 were also incubated for 4hrs in assay buffer (+ 10 mM  $\text{MgCl}_2$ ) at  $37^{\circ}\text{C}$ . These tests were negative for the formation of linear products, showing the absence of nuclease contamination.

### Reference

Stewart, L., Ireton, G.C., Parker, L.H., Madden, K.R. and Champoux, J.J. (1996). Biochemical and biophysical analyses of recombinant forms of human topoisomerase I. *J. Biol. Chem.* 271: 7593-7601