Human Topoisomerase I

Product Description (Product Numbers HT101, HT105, HT110 and HT120)

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 minimum concentration of 5 – 10 U/µl in Dilution Buffer. However, we recommend that the enzyme is titrated into the assay to ascertain the minimum volume of enzyme required per assay to achieve full supercoiling. Particularly if the kit is being used for drug screening purposes. Please refer to the protocol for more information:
https://www.inspiralis.com/assets/TechnicalDocuments/Human-Topo-I-Relaxation-Assay-Protocol3.pdf

Store at -80 °C. It is recommended that the enzyme is aliquoted to avoid repeated freeze-thaw cycles.

For *in vitro* laboratory research use only.

Dilution Buffer

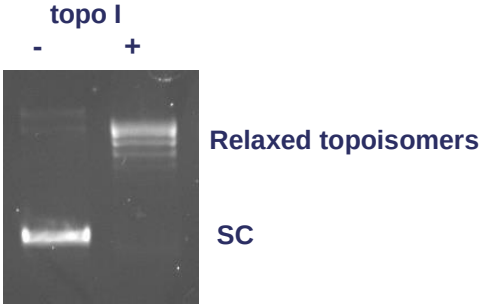
10 mM Tris.HCl (pH 7.5)
1 mM DTT
1 mM EDTA
50 % (v/v) glycerol
50 µg/ml albumin

Assay Buffer
(supplied as 10X stock)

20 mM Tris.HCl (pH7.5)
200 mM NaCl
0.25 mM EDTA
5 % glycerol
50 µg/ml albumin

Relaxation Assay

1 U of topoisomerase I will relax 0.5 µg of supercoiled pBR322 when incubated in Assay Buffer in a total reaction volume of 30 µl at 37 °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 $MgCl_2$) at 37 °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