

# Human Topoisomerase I Assay Kit For Cell Extracts



## Product Description (Product Numbers TRAK1, TRAK2 and TRAK3)

The kit is designed for use with human topo I or for the detection of human topo I in overexpressed cells. The kit contains 10X assay buffer, dilution buffer, supercoiled plasmid DNA substrate (sufficient for 100 assays) and relaxed DNA control.

Store at - 20 °C.

**For *in vitro* laboratory research use only.**

### Dilution Buffer

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

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

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

### Relaxed DNA Marker

Relaxed pBR322 in 1X loading dye

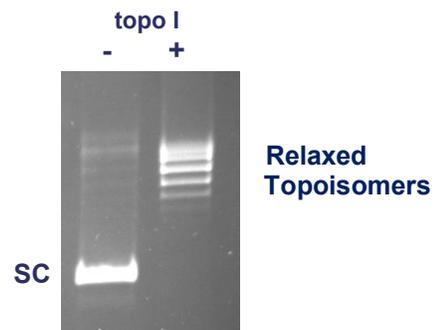
Supplied at 20 ng/µL

Suggested loading: 10 µL/well = 200 ng

### 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.

(For cell extracts use increased dilutions to avoid contaminating nuclease activity)



### 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