



## ProFoldin

10 Technology Drive, Suite 40  
Hudson, MA 01749-2791 USA  
FAX: (508) 845-9258  
[www.profoldin.com](http://www.profoldin.com)  
[info@profoldin.com](mailto:info@profoldin.com)

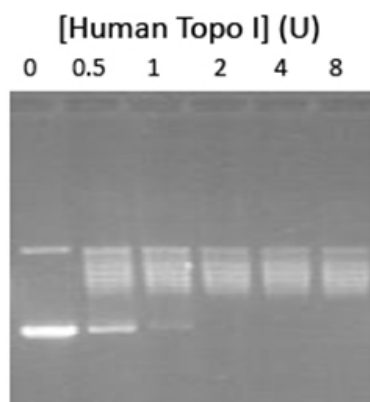
# INSTRUCTIONS

## ProFoldin Human Topoisomerase I

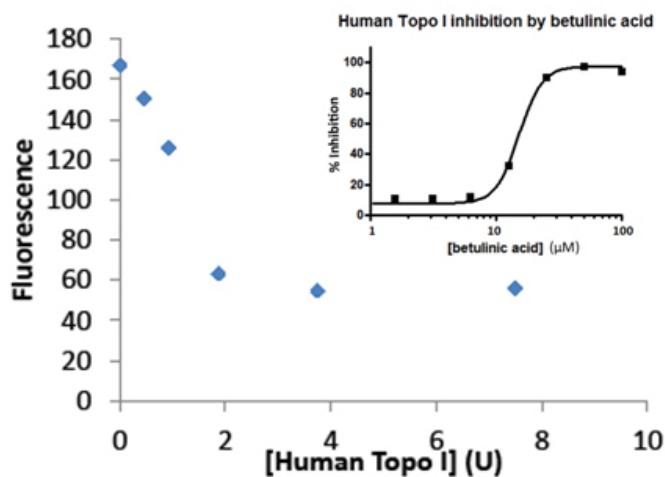
|   |                               |
|---|-------------------------------|
| <b>Human Topoisomerase I, 10,000 Units</b>                    | <b>Catalog No. HTOPI-010</b>  |
| <b>Human Topoisomerase I, 40,000 Units</b>                    | <b>Catalog No. HTOPI-040</b>  |
| <b>Human Topoisomerase I, 100,000 Units</b>                   | <b>Catalog No. HTOPI-100</b>  |
| <b>10 x Assay Buffer for Human DNA Topoisomerase I Assays</b> | <b>Catalog No. HT1BUF-100</b> |

|                       |   |
|-----------------------|---|
| Protein construct:    | Wild-type human topoisomerase I purified from a baculovirus insect protein expression system.   |
| MW:                   | 92 kDa, single subunit  |
| Enzyme concentration: | 500 U / $\mu$ l for HTOPI-010; 2000 U / $\mu$ l for HTOPI-040 and HTOPI-100.  |
| Enzyme Unit:          | One unit is the enzyme amount that relaxes 0.25 $\mu$ g of supercoiled plasmid DNA in 30 minutes at 37 °C.  |
| DNA Relaxation assay: | Use 4 U of Topoisomerase I and 1 $\mu$ g of supercoiled DNA for DNA relaxation assays at 37°C. Use a higher enzyme concentration if the reaction temperature is room temperature. The DNA relaxation activity is detected by agarose gel electrophoresis or 96-well plate fluorescence assay. Human DNA relaxation assay kit is available from ProFoldin (Catalog No. HRA100K for 100 assays). Reagents for HTS quantities are available. |
| Storage temperature:  | 4°C for short term or -80°C for long term storage. Do not freeze-and-thaw repeatedly.   |
| Enzyme dilution:      | Use the 1 x Buffer HT1 to dilute the enzyme just before the assay. Do not store diluted enzyme solution.  |

**Agarose gel assay format**



**96-well plate format**





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**Human Topoisomerase I, 10,000 Units (Catalog No. HTOPI-010)** contains 20 µl of 500 U/µl of purified human topoisomerase I.

**Human Topoisomerase I, 40,000 Units (Catalog No. HTOPI-040)** contains 20 µl of 2,000 U/µl of purified human topoisomerase I.

**Human Topoisomerase I, 100,000 Units (Catalog No. HTOPI-100)** contains 50 µl of 2,000 U/µl of purified human topoisomerase I.

**10 x Assay Buffer for Human DNA Topoisomerase I Assays (Catalog No. HT1BUF-100)** contains 100 ml of 10 x assay buffer (10 x Buffer HT1) for human topoisomerase I assays.

## DNA Relaxation Assay Protocol using the Human Topoisomerase I DNA Relaxation Assay Kit

### 1. Reaction and sample preparation:

The total volume of each reaction mixture is 40 µl including: 28 µl of H<sub>2</sub>O, 4 µl of 10 x Buffer HT1, 4 µl of 10 x supercoiled DNA (250 µg/ml), 4 µl of 10 x enzyme (1000 U/ml). Incubate the reaction mixture at 37°C for 60 min.

Note: The final concentrations are 25 mM Tris-HCl, pH 7.4, 58 mM KCl, 0.25 mM DTT, 5 mM MgCl<sub>2</sub>, 0.25 mM EDTA, 15 µg/ml BSA, 25 µg/ml supercoiled plasmid DNA and 100 U/ml topoisomerase I. A negative control reaction can be the reaction mixture without the enzyme.

### 2. Assay

- (1) For each 10 assays, freshly dilute 2 µl of 1500 x H19 dye into 3 ml of 1x H19 dilution buffer. The 1x H19 dilution buffer is prepared by 10 fold dilution of the 10x H19 dilution buffer with water.
- (2) Mix 250 µl of the freshly prepared 1 x H19 dye with each reaction solution (40 µl). Incubate the mixture at room temperature for 5 min.
- (3) Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm.

### Related Products:

Human topoisomerase I DNA relaxation assay kit plus-100  
Human topoisomerase I DNA relaxation assay kit plus-1000  
Supercoiled Plasmid DNA -1 mg  
Relaxed Plasmid DNA -1 mg  
H19 Dye for DNA Relaxation and Supercoiling Assays  
*E. coli* DNA Topoisomerase I Assay Kit Plus-100

Catalog No. HRA100KE  
Catalog No. HRA1000KE  
Catalog No. SDNA-1MG  
Catalog No. RDNA-1MG  
Catalog No. DSA1000D  
Catalog No. DRA100KE

For more information of DNA topoisomerase assays and assays for other drug targets and enzymes, please visit [www.profoldin.com](http://www.profoldin.com) or send emails to [info@profoldin.com](mailto:info@profoldin.com).