



Product Description

Contains purified a catalytically active wild type human topoisomerase I purified to homogeneity (single band on SDS-PAGE). The enzyme has been overexpressed in baculovirus and affinity purified as a single band on SDS-PAGE of 100kDa. The final preparation of enzyme is highly active in relaxation of plasmid DNA.

Storage and Shipping Conditions

The enzyme is shipped on dry ice and should be stored at -20°C . The enzyme has a useable lifetime of about 6 months when stored under optimal conditions. Note that repeated freezing and thawing will accelerate loss. The enzyme is provided at a unit/ul concentration that represents a certified minimum. For example, we certify that the product will have X units/ul under conditions of our assay. In some cases, activity may be greater than X units to take into account potential loss during shipping and handling of product. The enzyme will retain the certified minimum unit concentration (see label) for 6 months after receipt.

Unit Definition

One unit of Topoisomerase I can relax 0.25 ug (250 ng) of supercoiled plasmid DNA in 30 min at 37°C .

Quality Control Tests

1. A test for nuclease contamination was carried out by assaying for the formation of linear KDNA and linear plasmid DNA. Incubations of 1 μg of catenated KDNA or supercoiled pUC19 DNA (4 hrs. at 37°C in the presence of 10 mM MgCl_2) were performed. Linear DNA or breakdown products were not generated under these conditions.
2. A check for cross contamination with topo II was negative. There was no decatenation of KDNA in topo II reaction conditions.
3. The final fraction of topoisomerase I is a column pool and is in the following buffer: 20mM NaH_2PO_4 (pH7.4), 300mM NaCl, 50ug/ml BSA, 50% glycerol, 50mM Imidazole. The final fraction was analyzed by SDS-PAGE and shown to contain a single, predominant band of 100 kDa (Fig. 1).
4. Activity assays. Relaxation assays were carried out in a final volume of 25 μl in topo I reaction buffer (10X reaction buffer--also called TGS--supplied with this product is: 100 mM Tris-Cl (pH 7.9), 10 mM EDTA, 1.5 M NaCl, 1% BSA, 1 mM Spermidine, 50% glycerol). Supercoiled plasmid DNA was included at 0.25 μg /reaction. Reactions are terminated with 5 μl (per 20 μl reaction volume) of stop buffer (5% sarkosyl, 0.0025% bromophenol blue, 25% glycerol). Reaction products are analyzed on a 1% native agarose gel (no ethidium bromide in the gel or buffer during electrophoresis). A typical activity titration is shown in Fig 2.

Dilution Buffer

0.25ml Topo I dilution buffer is provided (20 mM NaH_2PO_4 pH 7.4, 300 mM NaCl).

Product Application and Disclaimer

This product is not licensed or approved for administration to humans or animals. It may be used with experimental animals only. The product is for in vitro research diagnostic studies only. The product is non-infectious and non-hazardous to human health. This information is based on present knowledge and does not constitute a guarantee for any specific product features and shall not establish a legally valid contractual relationship. TopoGEN, Inc. shall not be held liable for product failure due to mishandling and incorrect storage by end user. TopoGEN's liability is limited to credit or product replacement.

Figure 1. SDS PAGE analysis of WT human topo I.

Topo I was purified from baculovirus infected cells containing overexpressed human topo I with a his-6 tag. The final topo I fraction was analyzed using SDS-PAGE commassie staining.

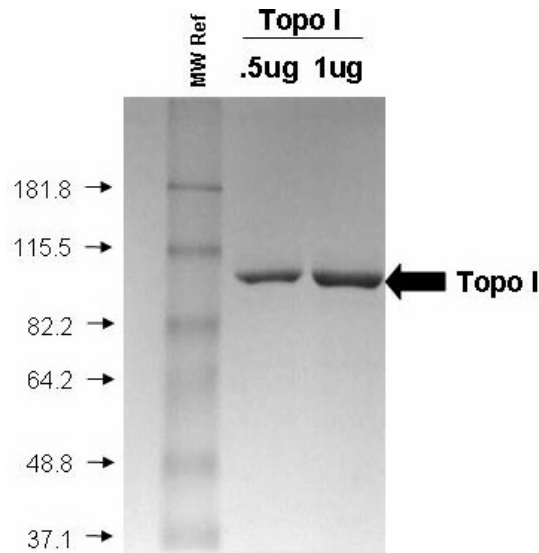


Figure 2. Titration of recombinant human topo I activity.

Topo I as prepared and purified using the baculovirus overexpression system. Activity was determined by serial 2 fold dilutions using pBR322 supercoiled DNA (30 min. at 37°C). The data show that this preparation titrates to approximately 32 units/ul.

