



Product Description

Contains purified a catalytically inactive mutant form of human topoisomerase I purified to homogeneity (single band on SDS-PAGE). The mutation is a single residue change at the active site tyrosine (which has been changed to phenylalanine). This preparation is overexpressed in baculovirus and affinity purified as a single band on SDS-PAGE of 100 kDa. The enzyme cannot relax DNA (but still binds to DNA) and contains a short (6 residue) histidine tag.

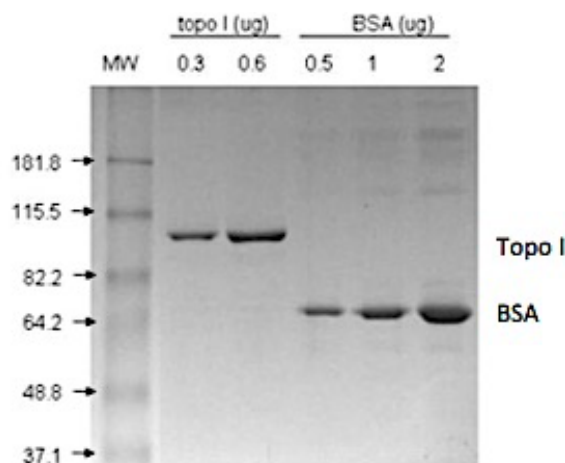
Storage and Shipping Conditions

The mutant enzyme is shipped on wet ice (dry ice to international locations) and should be stored at -20°C. The mutant enzyme is catalytically inactive and cannot relax supercoiled DNA.

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° in the presence of 10 mM MgCl₂) 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 NaH₂PO₄ (pH7.4), 300mM NaCl, 500mM 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 20-30 µl in topo I reaction buffer (10X reaction buffer, supplied with this product is: 100 mM Tris-Cl, pH 7.9, 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. Under these conditions, relaxation activity was not detectable with up to 0.5 ug of mutant protein.

Figure 1. SDS PAGE Analysis of Mutant Topo I
 Topo I was purified from baculovirus infected cells containing overexpressed human topo I with a his-6 tag. The mutant topo I was analyzed with SDS-PAGE and commassie staining.



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.