

Human Topoisomerase I (baculovirus expressed) Cat# 2005H-RC1

Description:

TopoGEN now offers a high purity and catalytically active human topoisomerase I overexpressed in the baculovirus system and purified to single band homogeneity. These preparations are cheaper than native topo I made from human tissue and offer excellent purity and high specific activity. In addition, the 100 kDa form of topo I is stable and is less prone to proteolysis, relative to the native protein from human cells.

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 μ g 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 below).

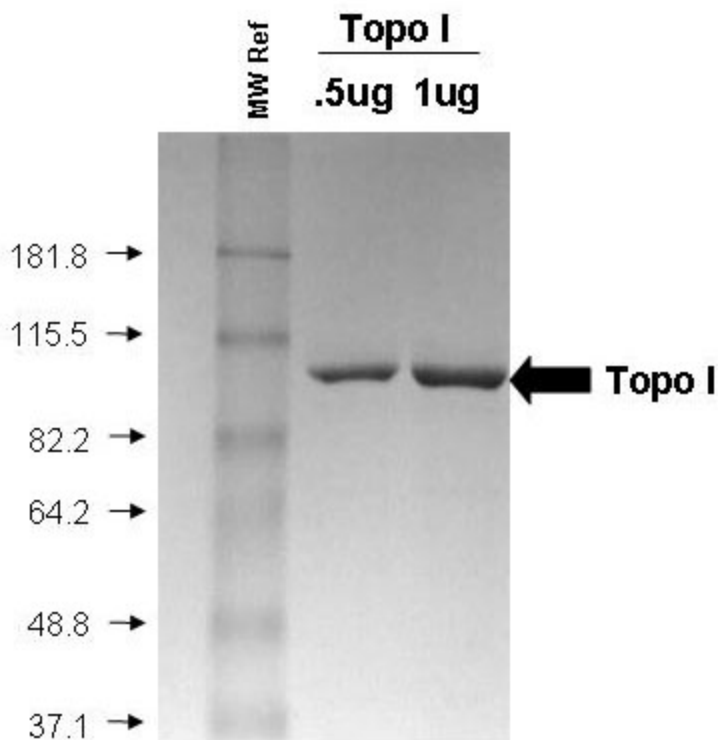


Figure 1

Activity assays:

Relaxation assays were carried out in a final volume of 25 μ l 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 μ g/reaction. Reactions terminated with 5 μ l (per 20 μ l reaction volume) of stop buffer (5% sarkosyl, 0.0025% bromophenol blue, 25% glycerol). Reaction products were analyzed on a 1% native agarose gel. Under these conditions, relaxation activity is easily detectable (typically we see 10-40 units of activity per μ l). A typical titration is shown in Fig. 2 .

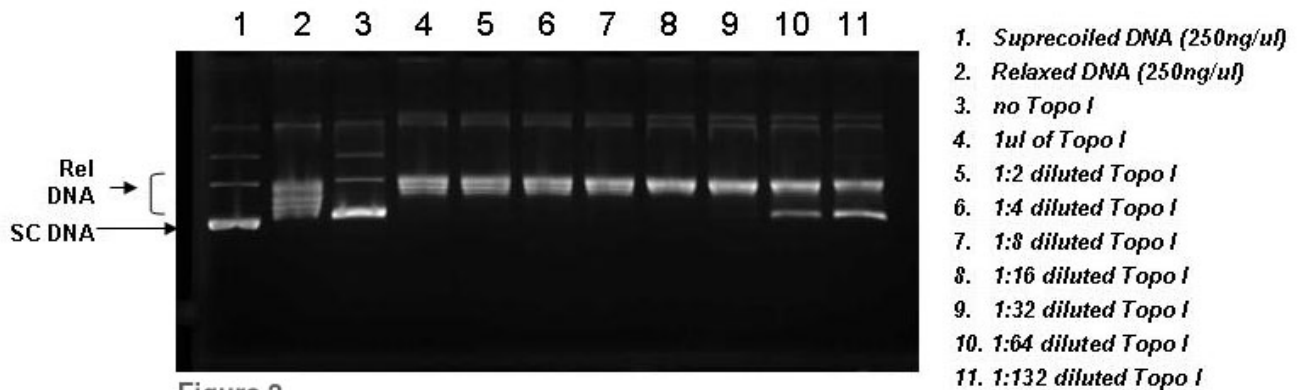


Figure 2