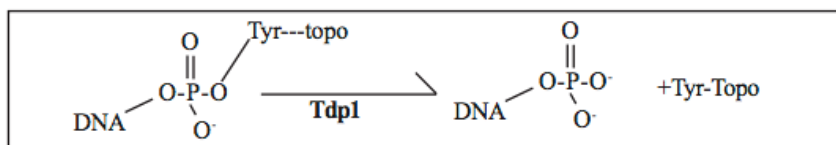




Product Description

Contains purified Human Tyrosyl DNA Phosphodiesterase (Tdp1) purified to homogeneity to a single 68 kDa band on SDS-PAGE. Tdp1 is catalytically active and will remove a terminal 3' phosphotyrosyl group from a specifically designed oligo target site (oligo-Tyr).



Storage and Shipping Conditions

The enzyme is shipped on wet ice and should be stored at -20°C. The enzyme has a useable lifetime of about 6 months even when stored under optimal conditions at -20°C. Note that repeated freezing and thawing will accelerate loss. **IMPORTANT NOTE REGARDING ACTIVITY:** 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 that freezing/thawing may lead to some loss over time. The enzyme will retain the certified minimum unit concentration (see label) for 6 months after receipt.

Unit Definition

One unit of Tdp1 can release the terminal Tyrosine from 20 ng of substrate (oligo-Tyr) in 30 min at 37°C.

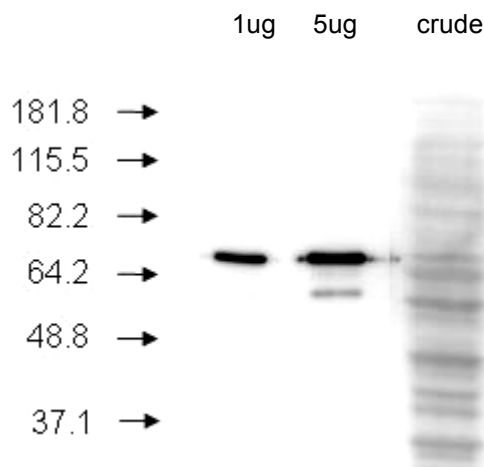


Fig. 1. SDS-PAGE Analysis of Tdp-1

Tdp-1 was purified by affinity chromatography and loaded onto a 7.5% SDS-PAGE gel. A normal load (1ug) and an overloaded lane (5ug) of the purified fraction is shown. The minor sub-band in the 5ug load is a small amount of break-down product (reacts with anti-Tdp-1 antibody).

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.

Human Tdp1 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 MgCl₂) were performed. Linear DNA or breakdown products were not generated under these conditions.
2. A check for cross contamination with topoisomerase activity was negative. There was no decatenation of KDNA in topo II reaction conditions nor was any topo I relaxation activity detected in the final enzyme fraction.
3. The final fraction of Tdp1 was analyzed by SDS-PAGE as shown in Fig. 1. A single prominent band is seen with a small amount of breakdown product. It is in the following buffer: 50 mM Tris-Cl, pH 7.5, 50mM KCl, 1 mM EDTA, 2 mM dithiothreitol, 50% glycerol. The enzyme is stable in this buffer at -20°C.
4. The Tdp-1 is enzymatically active. Typically, 1 ng of Tdp-1 will completely uncouple Tyrosine from 20 ng of the oligo-Tyrosine substrate (this equals one unit). The label with this product defines the units of activity.

Dilution Buffer

Dilutions should be performed in 20 mM Tris-Cl (pH 7.5), 100 mM KCl, 1 mM DTT (assay buffer).

Assay Conditions

Tdp1 assays are carried out in a final volume of 10 µl in assay buffer (20 mM Tris-Cl (pH 7.5), 100 mM KCl, 1 mM DTT) and at least 1-5 units of Tdp1 enzyme. The oligo-Tyr (sold separately and available from TopoGEN in the Tdp1 Assay Kit, TG1004) should be 5' end labeled using T4 polynucleotide kinase and γ [³²P]-ATP. We recommend using about 1×10^4 to 2×10^4 CPM per reaction and approximately 5-25 ng of the oligo-Tyr substrate. After incubation for 30 min at 37°C, reactions are terminated with 10 µl of formamide loading buffer (96% formamide, 20 mM EDTA, 0.03% xylene cyanol and 0.03% bromophenol blue). Reaction products are analyzed on a 20 sequencing gel.

References

- Interthal, H., Pouliot, J. and Champoux, J., 2001. The tyrosyl-DNA phosphodiesterase Tdp1 is a member of the phospholipase D superfamily. PNAS 98: 12009-12014
- Yang, S., Burgin, A, Huizenga, B., Robertson, C, Yao, K., and Nash, H. 1996. A eukaryotic enzyme that can disjoin dead-end covalent complexes between DNA and type I topoisomerases. PNAS 93:11534-11539