Active Recombinant Human TDP1 Protein

Cat. No. TDP1-3328H **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Purified Recombinant Human Tyrosyl DNA Phosphodiesterase(Tdp1).
Species	Human
Source	E.coli
Description	The protein encoded by this gene is involved in repairing stalled topoisomerase I-DNA complexes by catalyzing the hydrolysis of the phosphodiester bond between the tyrosine residue of topoisomerase I and the 3-prime phosphate of DNA. This protein may also remove glycolate from single-stranded DNA containing 3-prime phosphoglycolate, suggesting a role in repair of free-radical mediated DNA double-strand breaks. This gene is a member of the phospholipase D family and contains two PLD phosphodiesterase domains. Mutations in this gene are associated with the disease spinocerebellar ataxia with axonal neuropathy (SCAN1).
Molecular Mass	68 kDa
Unit Definition	One unit of Tdp1 can release the terminal Tyrosine from 20 ng of substrate (oligo-Tyr) in 30 min at 37 centigrade
Usage	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 should be 5’ end labeled using T4 polynucleotide kinase and γ[32P]-ATP. We recommend using about 1 x10^4 to 2 x10^4 CPM per reaction and approximately 5-25 ng of the oligo-Tyr substrate. After incubation for 30 min at 37 centigrade,

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	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.
Quality Control Test	1. A test for nuclease contamination was carried out by assaying for the formation of linear KDNA and linear plasmid DNA. Incubations of 1µg of catenated KDNA or supercoiled pUC19 DNA (4 hrs. at 37 centigrade 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. 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 centigrade. 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.
Stability	The enzyme has a useable lifetime of about 6 months even when stored under optimal conditions at -20 centigrade. Note that repeated freezing and thawing will accelerate loss.
Storage	Store at -20 centigrade
Dilutions	20 mM Tris-Cl (pH 7.5), 100 mM KCl, 1 mM DTT (assay buffer)
Warning	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

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	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 for 6 months after receipt.

GENE INFORMATION

Gene Name	TDP1 tyrosyl-DNA phosphodiesterase 1 [Homo sapiens (human)]
Official Symbol	TDP1
Synonyms	TDP1; tyrosyl-DNA phosphodiesterase 1; tyrosyl-DNA phosphodiesterase 1; tyr-DNA phosphodiesterase 1
Gene ID	55775
mRNA Refseq	NM_001008744
Protein Refseq	NP_001008744
MIM	607198
UniProt ID	Q9NUW8

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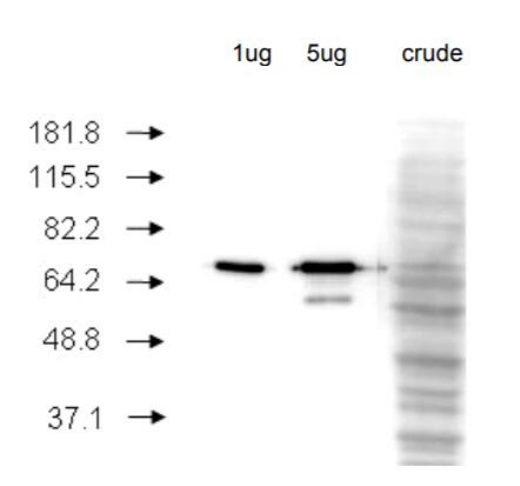
SDS-PAGE Analysis of Tdp-1



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

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