

Product Description

The vial contains Human Tyrosyl DNA Phosphodiesterase (Tdp2) purified to 80-90% homogeneity as a GST fusion protein, from *E. coli*. This protein appears to be multifunctional with overall similarity to APE-1 superfamily of Mg²⁺ and Mn²⁺ dependent phosphodiesterases. One important enzymatic function of TDP2 is the reversal of Top2/DNA covalent complexes that result from interfacial poisoning by drugs like etoposide and teniposide and many others. Knocking down cellular activity of TDP2 significantly enhances the sensitivity of the cell to Top2 interfacial poisons like etoposide, so the TDP2 role in recovery from Top2 mediated damage is very clear. [Detailed information on hu-TDP2 is available here.](#)

Catalytic Activity Assays, Unit Definition and Protein Purity.

One unit of Tdp2 will release 50ng of kDNA from a Top2/DNA complex in 30 min at 37°C.

Type of assay: TopoGEN prepares kDNA/Top2 complexes a known concentration (typically 200ng) which is incubated with Tdp2 for 30' at 37°C. Agarose gels are used to measure release of linear kDNA (Fig. 1). Fig 2 shows an SDS-PAGE gel of the affinity pure protein (lanes E1,E2 were pooled). Activity assays are shown (Fig. 3.)

Dilution Buffer: Dilutions should be performed in 20 mM Tris-Cl (pH 7.5), 100 mM KCl, 1 mM DTT or 1x Tdp2 assay buffer (see 'assay conditions'). Dilution buffer is not included.

Tdp2 Assays: Tdp2 is assayed under conditions optimized for hu-Top2 activity. Two buffers are included:

- Buffer A: 0.5 M Tris-HCl (pH 8), 1.50 M NaCl, 100 mM Mg₂Cl, 5 mM Dithiothreitol, 300 ug BSA/ml.
- Buffer B: 20mM ATP in sterile distilled water.

To prepare a fresh stock of the 5x Assay buffer: Add equal volumes Buffer A and B (1:1) to give the Complete 5x Assay Buffer (example, if you need 50 ul of 5x Complete Buffer for a single experiment, mix 25 ul of Buffer B with 25 ul of Buffer A). The Complete 5x Buffer MUST be made fresh for each experiment. Prepare only the amount needed fresh each day. This diluted 5x buffer should not be saved, as it is unstable.

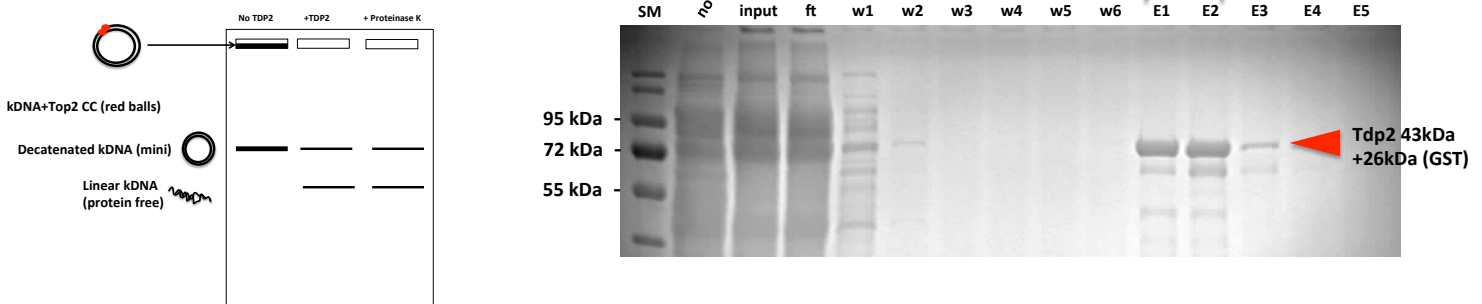


Fig. 1. TDP2 Assay Scheme. kDNA is reacted with huTop2a under conditions where the covalent complex (cc) is favored. This DNA is then used to assay for TDP2. Left most lane shows the substrate containing decatenated mini-circular DNA plus trapped Top2/DNA cc. These Top2/DNA cc contain linear DNA that cannot be resolved due to the trapped protein. The assay measures the release of the protein, which releases linear DNA into the gel.

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.

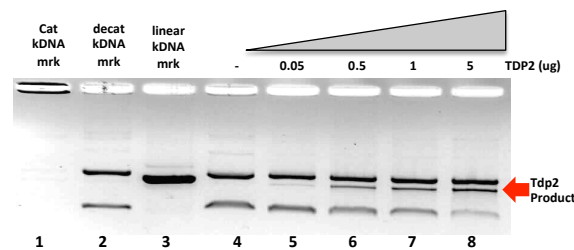


Fig. 3. Assay data. Purified Tdp2 (0 to 5ug) was incubated with kDNA/Top2 complexes (TG2038) under standard assay conditions. Reactions were terminated with SDS and analyzed on a 1% agarose gel. The linear kDNA product, released from the complex, is marked by a red arrow on the right.

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.

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