

TDP2 Assay Kits



Inspiralis

Product Description (Product Numbers TDP201, TDP205)

Human tyrosyl-DNA phosphodiesterase 2 (TDP2) assay kits are used to follow the activity of the enzyme TDP2. The assay follows the phosphotyrosyl hydrolysing activity of human TDP2 using a fluorescently-tagged phosphotyrosine oligo (5'-(6-FAM NHS)(5'-pTyr)GATCT(3'-BHQ-1)-3') as the substrate. The fluorescence of the FAM-tag is quenched by the 3'-BHQ tag in the substrate but the enzyme, TDP2, cleaves the phosphotyrosine bond separating the FAM and BHQ leading to an increase of the fluorescent signal at 520 nm (excitation at 485 nm). The enzyme is over expressed in *E. coli* and purified by methods developed in-house. The enzyme is supplied in Dilution Buffer. Store at -80 °C. It is recommended to avoid repeated freeze-thaw cycles. If this is likely then the enzyme should be aliquoted.

For *in vitro* laboratory research use only.

Dilution Buffer (Supplied as 1X Buffer)

25 mM Tris-HCl pH (7.5)
100 mM NaCl
1 mM DTT
50% Glycerol (v/v)

Assay Buffer (Supplied as 10X Buffer)

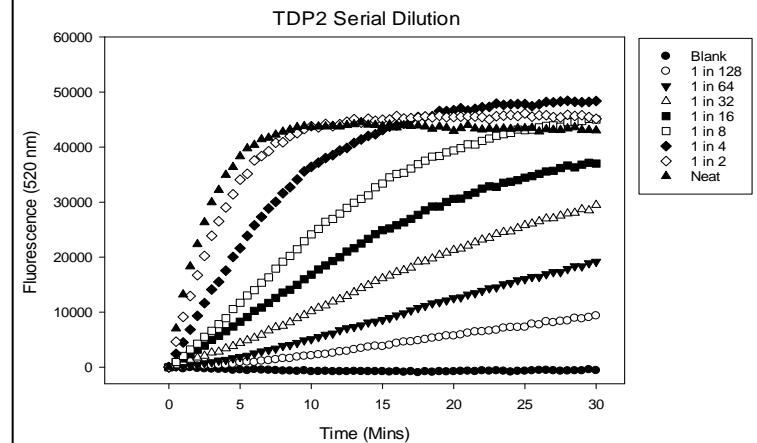
50 mM Tris.HCl (pH 7.5)
10 mM MgCl₂
80 mM KCl
0.05% (v/v) Tween-20
1 mM DTT

Substrate

5'-(6-FAM NHS)(5'-pTyr)GATCT(3'-BHQ-1)-3'.
Supplied at 4 μM in water

Fluorescence Assay

Assays are performed in a 20μL volume with a final substrate concentration of 1 μM. One unit of activity is defined as the amount of TDP2 to hydrolyse the substrate in 30 minutes at 37°C. In the example shown the amount of enzyme giving 1U of activity is at a 1 in 8 dilution equivalent to 0.125 μL.



Quality Control

Purity: The enzyme is purified to >95% purity as judged by SDS-polyacrylamide gel electrophoresis.

Endonuclease assay: 0.25 μg supercoiled pBR322 incubated with a >100-fold excess of TDP2 across its activity range for 30 mins and 3 hours at 37°C shows no detectable conversion of superhelical DNA to either open circular or linear forms when assayed by agarose gel electrophoresis.

Reference

Carlos *et al* (2018). New fluorescence-based high-throughput screening assay for small molecule inhibitors of tyrosyl-DNA phosphodiesterase 2 (TDP2). *Eur J Pharm Sci.* 2018 Jun 15; 118: 67–79.