

## Recombinant NgTet1 (1-321) protein

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**Catalog No:** 81148, 81848

**Expressed In:** *E. coli*

**Quantity:** 100, 1000 µg

**Concentration:** 0.8 µg/µl

**Source:** Naegleria

**Buffer Contents:** Recombinant NgTet1 protein is supplied in 25 mM Tris pH 8.0, 300 mM NaCl, 10% glycerol, and 0.5 mM TCEP.

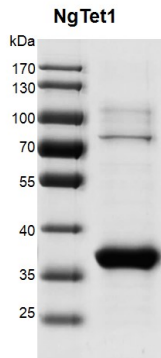
**Background:** Ten Eleven Translocation (TET) family proteins are cytosine oxygenases that catalyze the conversion of 5-methylcytosine (5-mC) into 5-hydroxymethylcytosine (5-hmC). 5-hmC can be further oxidized into 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC) by TET proteins. Methylation at the C5 position of cytosine is an epigenetic modification of the mammalian genome that plays an important role in transcriptional regulation. TET proteins are responsible for initiating the enzymatic deamination process leading to cytosine demethylation. The Tet family of dioxygenases is widely distributed across the tree of life, including in the heterolobosean amoeboid flagellate *Naegleria gruberi*. NgTet1 (*Naegleria* Tet-like protein) is a homologue of mammalian Tet proteins. Like mammalian Tet proteins, NgTet1 acts on 5-mC and generates 5-hmC, 5-fC and 5-caC.

**Protein Details:** Recombinant NgTet1 protein was expressed in *E. coli* cells as the full length protein (accession number XP\_002667965.1) with an N-terminal 6xHis Tag. The molecular weight of NgTet1 is 38.7 kDa.

**Application Notes:** Recombinant NgTet1 protein is suitable for use in cytosine oxygenase assays, 5-mC sequencing, enzyme kinetics, inhibitor screening, and selectivity profiling..

**TET Activity Assay Conditions:** 250 nM of 30bp DNA oligos (sequence: 5'-CAGTAACTGTGGTC(mC)GGTAACTGACTTGCA-3', contains a 5mCpG site) was incubated with 100 nM recombinant TET1 (1418-2136) protein in buffer containing 50 mM HEPES, pH 8.0, 100 µM Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>, 2 mM ascorbate, 1 mM alpha-ketoglutarate, 1 mM ATP, 1 mM TCEP at 37C for 1 hr. Activity was detected by Dot-blot.

**Storage and Guarantee:** Recombinant proteins in solution are temperature sensitive and must be stored at -80°C to prevent degradation. Avoid repeated freeze/thaw cycles and keep on ice when not in storage. This product is for research use only and is not for use in diagnostic procedures. This product is guaranteed for 6 months from date of arrival.



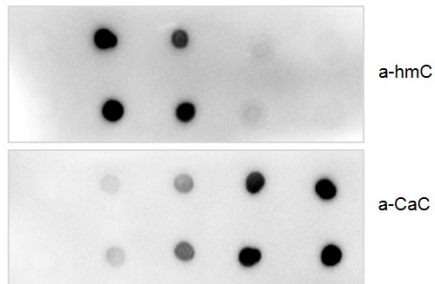
**Recombinant NgTet1 protein**

10% SDS-PAGE Coomassie staining

MW: 38.7 kDa

Purity: >85%

NgTet1 (ug)	0	0.5	1	2	4
mC-DNA (ug)	0	1	1	1	1



**Recombinant NgTet1 activity assay**

1  $\mu$ g of 30 nt ssDNA oligos with a 5mCpG site were incubated with different concentrations of NgTet1 in a reaction system at 34°C for 1 hour. Sample was concentrated to 10  $\mu$ l, and 1  $\mu$ l was spotted onto nylon membrane. 5hmC Ab (Cat. No. 39769) and 5-caC Ab (Cat. No. 61225) were used to detect products. NgTet1 activity assay was detected by Dot-Blot.