

TET Hydroxylase Activity Quantification Kit (Colorimetric) ab156912

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Overview

Product name	TET Hydroxylase Activity Quantification Kit (Colorimetric)
Detection method	Colorimetric
Sample type	Nuclear Extracts, Purified protein
Assay type	Enzyme activity
Sensitivity	> 20 ng
Assay time	5h 00m
Species reactivity	Reacts with: Plants, Mammals, Fungi
Product overview	TET Hydroxylase Activity Quantification Kit (Colorimetric) (ab156912) is suitable for measuring the activity/inhibition of total 5mC hydroxylase TET enzyme using nuclear extracts or purified TET isoforms (TET 1-3) from a broad range of species such as mammalian, plant, fungal, and bacterial, in a variety of forms including, but not limited to cultured cells, fresh and frozen tissues.

Notes

DNA methylation occurs by the covalent addition of a methyl group at the 5-carbon of the cytosine ring by DNA methyltransferases, resulting in 5-methylcytosine (5mC). In somatic cells, 5mC is found almost exclusively in the context of paired symmetrical methylation of the dinucleotide CpG, whereas in embryonic stem (ES) cells, a substantial amount of 5mC is also observed in non-CpG contexts. The biological importance of 5mC as a major epigenetic modification in phenotype and gene expression has been recognized widely.

5-hydroxymethylcytosine (5hmC), as a sixth DNA base with functions in transcription regulation, has been detected to be abundant in human and mouse brain and embryonic stem (ES) cells. In mammals, it can be generated by oxidation of 5mC, a reaction mediated by the ten-eleven translocation (TET) family of 5mC-hydroxylases.

The TET family of 5mC hydroxylases includes TET1, TET2 and TET3. These TET proteins may promote DNA demethylation by binding to CpG-rich regions to prevent unwanted DNA methyltransferase activity, and by converting 5mC to 5hmC and further to 5-carboxylcytosine (5-caC) through hydroxylase activity. It was shown that genomic 5hmC level correlates to TET hydroxylase activity. In addition, TET1 was shown to have dual functions in transcription activation and repression by binding different target genes in ES cells. TET1 is also a fusion partner of the MLL gene in acute myeloid leukemia and is considered an oncoprotein. TET2 is found to be frequently mutated in leukemia and considered to act as tumor suppressor. TET3 has been demonstrated to play a unique role for DNA methylation reprogramming processes in the mammalian zygote. Thus, activating tumor suppressor TET enzymes such as TET2 or inhibiting

oncoprotein TET enzymes such as TET1 would be important in benefiting cancer diagnostics and developing new target-based cancer therapeutics.

Platform Microplate reader

Properties

Storage instructions Please refer to protocols.

Components	48 tests	96 tests
10X TET Substrate	1 x 10µl	1 x 20µl
10X Wash Buffer	1 x 14ml	1 x 28ml
8-Well Assay Strips (with Frame)	1 x 6 units	1 x 12 units
Binding Solution	1 x 5ml	1 x 10ml
Capture Antibody, 1000 µg/mL	1 x 4µl	1 x 8µl
Co-factor 1	1 x 25µl	1 x 50µl
Co-factor 2	1 x 25µl	1 x 50µl
Co-factor 3	1 x 25µl	1 x 50µl
Detection Antibody, 400 µg/mL	1 x 8µl	1 x 16µl
Developer Solution	1 x 5ml	1 x 10ml
Enhancer Solution	1 x 8µl	1 x 16µl
Stop Solution	1 x 3ml	1 x 6ml
TET Assay Buffer	1 x 3ml	1 x 6ml
TET Assay Standard, 20 µg/mL	1 x 10µl	1 x 20µl

Function Dioxygenase that catalyzes the conversion of methylcytosine (5mC) to 5-hydroxymethylcytosine (hmC). Plays a role in embryonic stem (ES) cell maintenance and inner cell mass (ICM) cell specification, possibly by participating in DNA demethylation. Specifically binds 5mC, a minor base in mammalian DNA found in repetitive DNA elements that is crucial for retrotransposon silencing and mammalian development. 5mC is present in ES cells and is enriched in the brain, especially in Purkinje neurons. The clear function of hmC is still unclear but it could constitute an intermediate component in cytosine demethylation. A role of hmC in DNA demethylation is supported by TET1 function in ES cell maintenance, which is required to prevent NANOG hypermethylation and maintain NANOG expression in ES cells.

Tissue specificity Expressed in fetal heart, lung and brain, and in adult skeletal muscle, thymus and ovary. Not detected in adult heart, lung or brain.

Involvement in disease Note=A chromosomal aberration involving TET1 may be a cause of acute leukemias. Translocation t(10;11)(q22;q23) with MLL. This is a rare chromosomal translocation 5' MLL-TET1

3'.

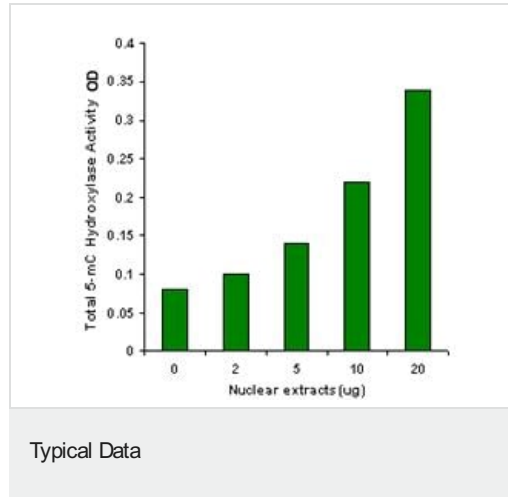
Sequence similarities

Belongs to the TET family.
Contains 1 CXXC-type zinc finger.

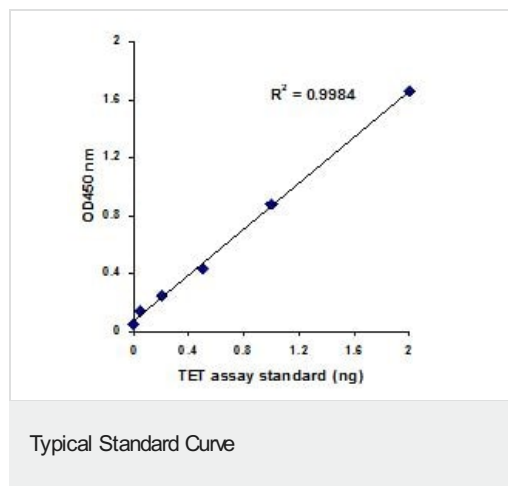
Cellular localization

Nucleus.

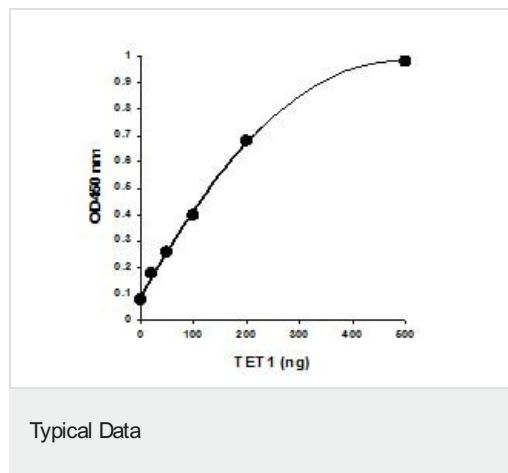
Images



Demonstration of high sensitivity and specificity of the TET activity assay achieved by using nuclear extracts with TET Hydroxylase Activity Quantification Kit (Colorimetric) (ab156912). Nuclear extracts were prepared from Mouse ES cells.



Illustrated standard curve generated with the TET assay standard.



Demonstration of high sensitivity and specificity of the TET1 activity/inhibition assay achieved by using recombinant TET1 with the TET Hydroxylase Activity Quantification Kit (Colorimetric) (ab156912).

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