TET2

Cat. No. : RK20557

Product components

Components	Component number	Size-1 10 RXN(50 μL/RXN)	Size-2 24 RXN(50 μL/RXN)
TET2	RM20540	40 μL	96 μL
TET2 Reaction Buffer	RM20815	300 μL	300 μL
TET2 Reaction Enhancer	RM20816	-	-
100 mM DTT	RM20117	10 μL	24 μL
Fe(II) Solution	RM20817	10 μL	10 μL

Product Description

TET2 is an enhanced methylcytosine dioxygenase, which has metal ion dependence and high oxidative activity. When combined with TET2 Reaction Buffer and Enhancer, TET2 catalyzes the oxidation of 5-methylcytosine (5-mC) into 5-hydroxymethylcytosine (5-hmC), so TET2 catalyzes the oxidation of 5-methylcytosine (5-mC) → 5-hydroxymethylcytosine (5-hmC) →5-formylcytosine (5-fC) →5-carboxycytosine (5-caC).

TET2 is a tool enzyme for methylation research, which is commonly used in NGS methylation detection, methylation PCR, and methylation qPCR. Compared with the traditional bisulfite treatment, the TET2 oxidation has less damage to DNA, which can maximize thef DNA integrity and sequencing quality. Such advantages specially lie in the methylation treatment of FFPE DNA, cfDNA, and other low-quality or trace DNA samples.

Store

-20℃ for long-term storage

Transportation

Dry ice , or dry ice combined with ice packs for Long-distance transportation. Do not use ice packs for long-distance transportation.

Precautions

1. About DNA Samples

1) The DNA sample should not contain EDTA, otherwise it will affect the TET2 reaction.

2) If the DNA sample is dissolved in a solution containing EDTA such as TE, it can be purified by purification beads and then eluted and transferred to nuclease-free water.

3) If user plans to input fragmented DNA, it is recommended to use ultrasonic fragmentation, rather than enzymatic fragmentation.

4) The average fragment sizes of 200-300 bp are recommended when DNA samples need to be fragmented.

2. About TET2 Reaction Enhancer

1) TET2 Reaction Enhancer is in a powder form, please centrifuge the powder to the tube bottom before use. Then add 300 μL of TET2 Reaction Buffer, fully dissolve and mix to be TET2 Buffer, which can be used directly.

2) The prepared TET2 Buffer needs to be labeled and dated, and the reagent needs to be stored in the dark to avoid repeated freeze-thaw cycles, and can be stored at -20℃ for 4 months.

3. About Iron Ions

1) Fe(II) Solution is used to activate the 5mC/5hmC oxidation.

2) Fe(II) Solution is freshly diluted to use. Discard it after use, do not store for next use.

Protocol

E-mail:info@abclonal.com

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1. Sample preparation

1.1. Prepare 10 ng-200 ng of EDTA-free dsDNA sample in 30 μL of Nuclease-free water in a clean PCR tube.

2. TET2 enzyme treatment

2.1. Add 300 μL of TET2 Reaction Buffer to the tube of TET2 Reaction Enhancer(powder), mix well and dissolve, spin down for few seconds, and place on ice for later use.

Note: TET2 Reaction Enhancer is powder, make sure to centrifuge to the tube bottom before use. The formulated TET2 Buffer can be stored at -20℃ for up to 4 months.

2.2. Prepare the following reaction on ice

Component	Volume
DNA sample (step 1.1.)	30 μL
TET2 Buffer ((step2.1.)	10 μL
DTT	1 μL
TET2	4 μL
Total	45 μL

Vortex to mix, instantaneously centrifuge to the bottom of the tube, and place on ice for later use.

2.3. Add 1 μL of Fe(II) Solution to 1249 μL of Nuclease-free water, vortex to mix, and spin down for few seconds.

Note: Fe(II) Solution is freshly diluted to use.

2.4. Prepare the reaction system as follows on ice

Component	Volume
Mix (step 2.2.)	45μL
Diluted Fe(II) Solution (step 2.3.)	5 μL
Total	50μL

Vortex to mix well and centrifuge instantaneously to the tube bottom.

2.5. Place the PCR tube on the thermal cycler, set the thermolid temperature to 50 ℃, and run the reaction program as follows:

Temperature	Time
37℃	1 h
4℃	Hold

Note:1. At the end of the reaction, use 1 μL of Proteinase K (20 mg/mL) (ABclonal, Cat.NO. RK20561) to teminate the reaction, and then use 1.8x magnetic beads (RK20257). to purify the terminated product.

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