APOBEC

Cat. No. : RK20559

Product components

Components	Component number	Size-1 10 RXN(100 μL/RXN)	Size-2 24 RXN(100 μL/RXN)
APOBEC	RM20542	10 μL	24 μL
APOBEC Reaction Buffer	RM20819	100 μL	240 μL
Recombinant Albumin (20 mg/mL)	RM20543	10 μL	24 μL

Product Description

Cytosine deaminase (APOBEC) has high deamination activity and plays an important role in the process of active DNA demethylation, which can remove the aminogroup of cytosine (C), 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) in single-stranded DNA to uracil (U).

APOBEC is a tool enzyme for methylation research, which is used in NGS methylation detection, methylation PCR, and methylation qPCR. Compared with traditional bisulfite treatment, mild enzymatic treatment has less damage to DNA, which can maximize the 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

Storage: -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.

Protocol

1. Sample preparation

1.1. Prepare 16 μL of sample containing 10 ng~200 ng of double-stranded DNA in a clean PCR tube.

2. DNA denaturation

2.1. Preheat the thermal cycler to 85 ℃ and set the heat lid temperature to 105 ℃.

2.2. Add 4 μL of formamide to the 16 μL DNA sample (step 1.1.), vortex to mix, and spin down for few seconds.

2.3. Place the PCR tube on a pre-heated thermal cycler and incubate at 85℃ for 10 min.

2.4. Transfer to ice immediately after the end of incubation and prepare for the deamination reaction.

3. Deamination reaction

3.1. Prepare the following reaction on ice:

Component	Volume
Deaminated DNA（Step 2.4.）	20 μL
APOBEC Reaction Buffer	10 μL
Recombinant Albumin (20 mg/mL)	1 μL
APOBEC	1 μL
Nuclease-free water	Up to 100 μL
Total	100 μL

Vortex to mix, spin down for few seconds.

3.2. Place the PCR tube on the thermal cycler, set the thermo lid temperature to 50 ℃, and run the following reaction program:

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Temperature	Time
37℃	3 h
4℃	Hold

Note: Reaction products can be purified with 1.8x magnetic beads (RK20257).

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