Fast Magnetic DNA Methylation Bisulfite Kit

Catalog No：RK30206

Components

Components Name	Components Number	Size-1 18 RXN	Size-2 50 RXN
Conversion Reagent	RM30514	1.5 mL × 3	1.5 mL × 7
Clean Beads	RM30515	300 μL	850 μL
Binding Buffer	RM30545	10 mL	28 mL
Wash Buffer	RM30516	5 mL	14 mL
Desulphonation Buffer	RM30517	4 mL	11 mL
Elution Buffer	RM30518	1 mL	1.8 mL

Product Description

This kit quickly converts unmethylated cytosines in DNA samples to uracil while leaving methylated cytosines unchanged. After bisulfite treatment at high temperature, double-stranded DNA is denatured into single strands. Under the action of HSO_3^-, cytosine residues undergo deamination and are converted to uracil, while methylated residues remain intact. Subsequently, during PCR amplification, uracil is replaced by thymine (T).

Storage

Store Conversion Reagent at 4 ~ 25℃ away from light (refrigeration is recommended). Other components should also be stored at room temperature (15 ~ 25℃).

Sample Requirements

- Sample types include DNA samples from exfoliated cells, paraffin-embedded tissue genomic DNA, and cell-free DNA from plasma.
- The input amount should be between 200 pg and 2 ug, and it is recommended to use DNA samples with a purity indicator of A260/A280 ratio between 1.7 and 1.9 for conversion.

Reagent Preparation

- Do not mix reagents from different batches. Avoid contact with skin and eyes when using Conversion Reagent/Desulphonation Buffer. If contact occurs, wipe off immediately and rinse with plenty of water. This kit does not provide anhydrous ethanol, which users need to prepare themselves.
- For 18 RXN: Add 20 mL of anhydrous ethanol to the Wash Buffer before use. After mixing, tighten the cap to prevent evaporation of ethanol affecting the reagent.
- For 50 RXN: Add 56 mL of anhydrous ethanol to the Wash Buffer before use. After mixing, tighten the cap to prevent evaporation of ethanol affecting the reagent.

Operation Instructions

Before starting the experiment, prepare the following reagents and consumables: 1.5 mL Nuclease-free centrifuge tubes, Nuclease-free pipette tips, Nuclease-free Water, anhydrous ethanol, 0.5 mL PCR tubes.

1. Bisulfite Conversion

1.1 Prepare sterile PCR tubes according to the number of samples needed and prepare the conversion reaction system as per the table below:

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Components	Volume
Input DNA	200 pg - 2 ug
Conversion Reagent	180 μL
Nuclease-free Water	Up to 200 μL
Total	**200 μL**

1.2 Mix the system well with a pipette or briefly vortex for 5 seconds, briefly centrifuge, and centrifuge the reaction liquid to the bottom of the PCR tube.

1.3 Execute the following conversion program: :

Temperature	Time
hot lid at 105℃	On
98℃	7 min
4℃	Hold

2. Conversion Product Purification

2.1 Before use, thoroughly vortex the Clean Beads to ensure homogeneity.

2.2 Add 15 μL of purified Clean Beads and the conversion product from the previous step into a 1.5 mL Nuclease-free centrifuge tube, then add 500 μL Binding Buffer. Mix well by pipetting or low-speed vortexing for 30 seconds, and then incubate at room temperature for 10 minutes.

2.3 Briefly centrifuge the centrifuge tube and place it on a magnetic stand. After the solution has cleared, carefully remove the supernatant.

Note: Use a pipette to thoroughly remove the supernatant.

2.4 Add 400 μL of Wash Buffer (which has had the specified volume of anhydrous ethanol added) to the centrifuge tube, mix well by pipetting or low-speed vortexing for 30 seconds. Briefly centrifuge and place on a magnetic stand. After the solution has cleared, carefully remove the supernatant.

Note: Add the Wash Buffer as soon as possible to avoid affecting the state of the magnetic beads.

2.5 Add 200 μL of Desulphonation Buffer to the centrifuge tube, mix well by pipetting or low-speed vortexing for 30 seconds. Incubate at room temperature for 10 minutes. Briefly centrifuge and place on a magnetic stand. After the solution has cleared, carefully remove the supernatant.

2.6 Add 400 μL of Wash Buffer (which has had the specified volume of anhydrous ethanol added) to the centrifuge tube, mix well by pipetting or low-speed vortexing for 30 seconds. Briefly centrifuge and place on a magnetic stand. After the solution has cleared, carefully remove the supernatant.

2.7 Repeat step 2.6 once more, discard the supernatant, and perform the wash twice in total.

Note: A 10 μL pipette can be used to remove residual liquid.

2.8 Dry at 55°C for 5 to 10 minutes or at room temperature for 10 to 20 minutes to thoroughly remove residual liquid in the tube, until the surface of the magnetic beads shows no reflection.

2.9 Add 30 μL of Elution Buffer, mix well by pipetting or low-speed vortexing for 30 seconds to fully reselect the magnetic beads, incubate at room temperature for 5 minutes, briefly centrifuge and place on a magnetic stand. After the solution has cleared, transfer the supernatant to a new 1.5 mL Nuclease-free centrifuge tube.

2.10 The conversion product can be stored at -30 to -15°C, and for long-term storage, it should be kept at -85 to -65°C, avoiding repeated freezing and thawing.

Precautions

- This kit is for research purposes only. Before starting the experiment, read this manual carefully.
- For smooth downstream experiments, it is recommended to use Qubit 3.0 or Qubit 4.0 to quantify the input DNA.
- The Conversion Reagent, Wash Buffer, and Desulphonation Buffer contain volatile components. After use, tighten the caps promptly and store at room temperature away from light.
- Ensure that the reagents are still within their validity period when used.

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