v

Fast Magnetic DNA Methylation Bisulfite

Product composition

Component name	Component number	Specifications-1 18 RXN	Specifications-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 product can rapidly convert unmethylated cytosine in DNA samples to uracil while maintaining methylated cytosine unchanged. After high-temperature bisulfite treatment of double-stranded DNA, the double strands separate into single strands. Under the action of HSO3, this leads to deamination of cytosine residues, converting them to uracil, while the methylated residues remain unchanged. In subsequent PCR amplification, uracil is then replaced by thymine (T).

Storage conditions

Conversion Reagent Components 4~ 25 should be stored in the dark (it is recommended to store in cold storage), and other components should be stored at 15 ~ 25 (room temperature).

Sample requirements

- Sample type: DNA samples that have not been transformed by CT, usually including genomic DNA from shed cells, the genome of tissue embedded in paraffin; and free DNA from plasma.
- Injection volume: 200 pg ~ 2 ug; it is recommended to use DNA samples with A260 / A280 ratio between 1.7 ~ 1. 9 for transformation.

Preparation of reagents

- Different batches of reagents are not recommended to be used together; Conversion Reagent / Desulphonation Buffer should be avoided from contact with skin and eyes during use, if accidentally contacted with skin or eyes, it should be immediately dried and washed with a large amount of water. The kit does not provide anhydrous ethanol, which should be prepared by the user.
- 18 RXN: Wash Buffer 20 mL anhydrous ethanol should be added when using the reagent. After adding, invert and mix the reagent. At this time, the bottle cap should be tightened to prevent the evaporation of ethanol from affecting the use of the reagent.
- 50 RXN: Wash Buffer Add 56 mL anhydrous ethanol when using. After adding, invert and mix. At this time, the bottle cap should be tightened to prevent ethanol evaporation from affecting the use of reagents.

operation declaration

Before the experiment, prepare the following reagents and consumables in advance: 1.5 mL Nuclease-free centrifuge tube, Nuclease-free gun head, Nuclease-free Water, anhydrous ethanol, 0.5 mL PCR tube.

1. Hydrogen sulfite conversion

1.1 Prepare the corresponding sterile PCR tubes according to the number of samples to be tested, and prepare the transformation reaction system according to the following table:

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

1.2 The above system was mixed by pipette or briefly vortexed for 5s, and then centrifuged briefly to remove the reaction solution to the bottom of the PCR tube.

1.3 Perform the following conversion procedure:

Temperature	Time
Hot cover 105	On
98℃	7 min
4℃	Hold

2. Purification of the transformed product

2.1 Shake the Clean Beads thoroughly before use;

2.2 Add 15 μL Clean Beads and the conversion product of the previous step to a 1.5 mL Nuclease-free centrifuge tube, add 500 μL Binding Buffer, blow with pipette or vortex at low speed for 30s to mix well, incubate at room temperature for 10 min;

2.3 After instantaneous centrifugation of the centrifuge tube, it was placed on the magnetic rack. After the solution was clarified, the supernatant was carefully removed;

Note: Use a pipette to remove the supernatant thoroughly.

2.4 Add 400 μL Wash Buffer (with specified volume of anhydrous ethanol) to the centrifuge tube, and mix thoroughly by pipette or low-speed vortex for 30s. After instantaneous centrifugation, place on a magnetic stand, and carefully remove the supernatant after the solution is clear;

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

2.5 Add 200 μL Desulphonation Buffer to the centrifuge tube and mix thoroughly by pipetting or low-speed vortexing for 30 s. Let the reaction sit at room temperature for 10 min. After instantaneous centrifugation, place on the magnetic frame and carefully remove the supernatant after the solution is clear;

2.6 Add 400 μL Wash Buffer (with specified volume of anhydrous ethanol) to the centrifuge tube, and mix thoroughly by pipette or low-speed vortex for 30s. After instantaneous centrifugation, place on the magnetic rack, and carefully remove the supernatant after the solution is clear;

2.7 Repeat step 2.6 once, discard the supernatant, and rinse twice in total;

Note: A 10 μL pipette can be used to suck up the residual liquid.

2.8 55 dry 5 ~ 10 min or room temperature dry 10 ~ 20 min, fully remove the residual liquid in the tube, dry until the magnetic bead surface is not reflective;

2.9 Add 30 μL of Elution Buffer, and resuspend the magnetic beads by pipetting or low-speed vortex for 30s. Incubate at room temperature for 5 min, then centrifuge briefly and place on a magnetic frame. After the solution is clear, transfer the supernatant to a new 1.5 mL Nuclease-free centrifuge tube;

2.10 The transformed products can be stored at-30 ~ -15 , and long-term storage should be placed at-85 ~ -65 , and repeated freezing and thawing should be avoided.

matters need attention

This kit is for scientific research only. Read this manual carefully before starting the experiment.

To ensure the smooth progress of downstream experiments, it is recommended to use Qubit 3.0 or Qubit 4.0 for the quantitative analysis of input DNA.

Conversion Reagent, Wash Buffer and Desulphonation Buffer contain volatile components. After use, the bottle cap should be tightened in time and stored in the dark. Conversion Reagent is recommended to be stored in cold storage.

Ensure that the reagent is still within its expiration date.