DNA Methylation Modification Kit

Cat: D2840

Size: 50T

Storage: Adsorption column DF store at 2-8℃, the rest of the components store at room temperature (15-30℃).

Kit Components:

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Converting solution CR	5mL	RT Light shelter
Buffer CL	30mL	RT
Buffer MD	0.4mL	RT
Buffer DB	10mL	RT
Washing buffer WB(concentrate)	10mL	RT
Washing buffer 1(concentrate)	13mL	RT
Washing buffer 2(concentrate)	15mL	RT
Elution Buffer	4mL	RT
Column balancing liquid PS	10mL	RT
Adsorption column DF	50 units	2-8℃
Collecting tubes	50 units	RT

Own reagents: anhydrous ethanol, PCR tube, EP tube, centrifuge.

Introduction:

The basic principle of this kit is that after DNA is treated with sodium bisulfite, unmethylated cytosine can be converted into uracil, while methylated cytosine remains unchanged.

This kit adopts alkaline denaturation treatment method, which can denaturate DNA double strand under mild conditions, ensure DNA integrity and improve the conversion efficiency, and the conversion efficiency can reach more than 99.5%. At the same time, by combining the transformation product with the adsorption column, the purified DNA can be recovered from the solution modified by bisulfite. The recovered DNA has high purity and good integrity, and can be directly used for sequencing, methylation PCR detection, chip analysis and other downstream experiments.

Product Advantages:

1. High conversion efficiency: unmethylated cytosine conversion efficiency is greater than 99.5%.
2. High nucleic acid integrity: little damage to nucleic acid, conducive to downstream detection.
3. High recovery rate: recovery rate is greater than 80%.
4. High sample compatibility: Compatible with 100pg-2μg of DNA, cfDNA.

Preparation and important notes before experiment:

1. Preparation before experiment: For the newly opened kit, add the corresponding amount of anhydrous ethanol to the washing buffer WB, washing buffer 1 and washing buffer 2 in advance according to the instructions on the reagent bottle label, and check whether anhydrous ethanol is added before use. After adding anhydrous ethanol, tighten the bottle cap to prevent volatilization.
2. Notes:
   1) Before the experiment, the specification should be read in detail, and the experiment operation should be carried out by personnel with professional experience and training.
   2) The conversion solution is sensitive to light and should be stored away from light to avoid exposure to light.
   3) This kit is suitable for DNA and cfDNA solutions extracted and purified from feces, blood, tissues, body fluids, etc. The amount of DNA injected in a single conversion is about 100pg-2μg, of which 10ng-2μg is better. At the same time, DNA should meet the A260/280 between 1.8 and 2.0. **However, it should be noted that** high input amount of DNA may lead to incomplete transformation of some high GC regions or highly structured DNA, and the input amount should be appropriately reduced according to the particularity of the sample.

Protocols:

1. Add 20μL sample to be tested and 5μL buffer MD into PCR tube, mix and centrifuge briefly, then put into PCR apparatus and incubate at 37℃ for 15min.
2. Add 100μL conversion solution CR, mix well and centrifuge briefly, then put into PCR instrument and incubate at 54℃ for 90min.

   Note: If the next experiment cannot be carried out in time after the end of incubation, hold at 4℃ can be set.
3. Add 200μL column balance liquid PS into the adsorption column that has been loaded into the collection tube, centrifuge at 12000rpm for 1min, discard the waste liquid, and put the adsorption column back into the collection tube.
4. Add 600μL buffer CL, transfer the transformed product obtained in step 2 to the adsorption column, turn it upside down 3-5 times, and leave it at room temperature for 10min.
5. Centrifuge at 12000rpm for 1min, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.
6. Add 500μL washing buffer1(check whether anhydrous ethanol has been added before use), centrifuge at 12000rpm for 1min, without discarding the waste liquid.
7. Add 200μL buffer DB, put at room temperature for 10-15min, centrifuge at 12000rpm for 1min, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

8. Add 500μL of washing buffer 2(check whether anhydrous ethanol has been added before use) and centrifuge at 12000rpm for 1min without discarding the waste liquid.
9. Add 200μL washing buffer WB(check whether anhydrous ethanol has been added before use), centrifuge at 12000rpm for 1min, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.
10. Centrifuge the collection tube with the adsorption column at 12000rpm for 2min.
11. Transfer the adsorption column(do not touch the inner wall of the collection tube in the process of transfer to prevent alcohol sticking) into a clean collection tube, open the cover, and let dry for 3min.
12. Add 20μL elution buffer to the middle membrane of the adsorption column(be careful not to poke the membrane of the adsorption column with the gun head, and change the gun head when adding different samples), cover the cap tightly, incubate at room temperature for 2min, centrifuge at 12000rpm for 1min, collect DNA solution, and set aside.

Notes:

1. If the recovery efficiency is to be improved, the eluent can be preheated at 65-70℃.
2. DNA modified by bisulfite is recommended to be tested immediately, otherwise please store below -20℃ for no more than 1 month, long-term storage should be placed at -70℃ or below, and repeated freeze-thaw should be avoided.

Common Problems:

1. Should the input DNA be dissolved in TE, water, or other buffers before conversion?

Water, TE, or modified TE buffers can be used to dissolve the DNA and do not interfere with the conversion process.

2. Can the methylation conversion time be prolonged?

In the process of methylation conversion, too long the conversion time will cause the methylated C part to become U, resulting in false negative, please strictly follow the operating instructions.

3. What do you need to pay attention to in DNA quantification of bisulfite treatment?

After genomic DNA is treated with bisulfite, the original base pairing is no longer present because the unmethylated cytosine residues are converted to uracil, and the recovered DNA is usually rich in A, U, and T, and is single stranded at room temperature with limited non-specific base pairing. The absorption coefficient at 260nm is similar to that of RNA. It is recommended that a value of 40μg/mL be used for Ab260=1.0 when detecting DNA concentration after treatment with bisulfite

4. Why is the OD value abnormal for nucleic acid detection after treatment?

Due to the particularity of nucleic acid state after treatment, OD230 will be abnormal, which may lead to the instability of the OD260/OD230 ratio. The experiment shows that this situation does not affect the subsequent PCR reaction. The OD260/OD280 ratio should be 1.7-1.9. If the

elution buffer is not used and ddH20 is used, the ratio will be low because the pH and the presence of ions will affect the light absorption value, but does not indicate low purity.

5. What are the recommended conditions for subsequent PCR amplification?

Typically, bisulfite transformed DNA requires 35 to 40 cycles for successful PCR amplification, and the optimal amplicon size should be between 150-300bp, but larger amplicons (up to 1kb) can be generated by optimizing PCR conditions, and annealing temperatures between 55-60 ° C usually work well. Since most non-methylated cytosine residues are converted to uracil, DNA treated with bisulfite is usually rich in AT, and because of this, non-specific PCR amplification is relatively common, and the use of "hot start" polymerase for PCR amplification of DNA treated with bisulfite is strongly recommended.