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Instructions for samples preprocessing kit for methylation test

Product name

Samples preprocessing kit for methylation test

Packing specification

Type I (magbead method) : 25 tests/box, 50 tests/box, 200 tests/box
Type II (column method) : 25 tests/box, 50 tests/box, 200 tests/box

Intended use

For bisulfite conversion modification, extraction, enrichment, purification steps of nucleic acids. The processed products are used for clinical genetic testing.

Principle

The basic principle of this kit is that DNA treated with bisulfite can convert unmethylated cytosine into uracil, while methylated cytosine remains unchanged. And using the original high temperature treatment method, greatly shorten the time of methylation conversion, improve the conversion efficiency, the conversion efficiency can be achieved more than 99%.This kit can recover DNA from methylation-modified solution with high purity and integrity, which can be directly used for sequencing and methylation PCR. This kit can be used in automated instruments for methylation conversion and purification, which can greatly reduce the work of laboratory personnel.

Main components

1. The reagent components contained in the kit.

Components	Specification						Main components
	Type Ⅰ			Type Ⅱ			
	25 tests/box	50 tests/box	200 tests/box	25 tests/box	50 tests/box	200 tests/box	
Conversion solution	1.5 mL×4	1.5 mL×8	1.5 mL×32	1.5 mL×4	1.5 mL×8	1.5 mL×32	Ammonium bisulfite
Buffer MB	10 mL×1	20 mL×1	80 mL×1	15 mL×1	30 mL×1	120 mL×1	Guanidine thiocyanate
Washing WB	10 mL×1	10 mL×2	40 mL×2	10 mL×1	10 mL×2	40 mL×2	Deionized water
Buffer DB	5 mL×1	10 mL×1	40 mL×1	5 mL×1	10 mL×1	40 mL×1	NaOH
Elution Buffer	1 mL×2	4 mL×1	8 mL×2	1 mL×2	4 mL×1	8 mL×2	Tris, EDTA
Magbead solution	0.5 mL×1	1 mL×1	1 mL×4	/	/	/	Magbead
Buffer PS	/	/	/	5 mL	10 mL	40 mL	NaCl
Adsorption column DF	/	/	/	25	50	200	/
Collection Tube	/	/	/	25	50	200	/

Note: Components in different models and batches of kits cannot be mixed.

2. The product does not include self-provided reagents and instruments
   Instruments: constant temperature mixer, Benchtop centrifuge, pipette (specifications: 10 μL, 100 μL, 200 μL, 1000 μL) Reagent consumables: experimental needs but not included in the kit absolute ethanol, tipswith filter element (specifications: 10 μL, 100 μL, 200 μL, 1000 μL), 1.5 mL centrifuge tube, 2 mL centrifuge tube, magnetic rod sleeve, 96 deep well plate.

Storage Conditions and Expiry Date

1. It can be transported at 4-37°C, and the transportation time is not more than 7 days.
2. The adsorption column was stored at 2-8°C, the converted liquid was sealed at -20±5°C and stored away from light, and the remaining components were stored at 4-30°C. The kit is valid for 12 months.
3. The conversion liquid is sensitive to light and easy to oxidize. After opening, it can be used for a single time, and the number of freezing and thawing is not more than 2 times.

   Note: If it is normal for the component solution to crystallize out, the use effect will not be affected after redissolution.

4. Buffer DB contains sodium hydroxide, which is moderately toxic. After use, the waste liquid shall be stored in the waste liquid bottle and handed over to the third party for professional treatment.
5. In the process of methylation conversion, too long conversion time will cause the methylated C part to become U, resulting in false negative. Please strictly follow the operation instructions.
6. This kit is only used for in vitro diagnosis. Please read the instructions carefully and follow the instructions strictly before use.
7. It should be operated by experienced or trained personnel.
8. The relevant laboratory management standards should be strictly implemented in accordance with the relevant gene amplification laboratory management standards issued by the administrative department of the industry.
9. Do not use reagents beyond the expiration date, and do not mix different batches of products.

98°C	10 min
54°C	1 h

3.3 Add the following reagents into the sample deep-well plate

Matches CWE2100:

Position	Add the reagent in a single well
1&7 columns	Buffer MB: 400 μL Isopropanol: 200 μL Post-conversion product: 220 μL Magbead solution: 20 μL
2&8 columns	Wasing WB：500 μL
3&9 columns	Buffer DB：200 μL
4&10 columns	Wasing WB：500 μL
5&11 columns	Washing WB：200 μL
6&12 columns	Elution Buffer：40-80 μL

Matches CWE960

Position	Add the reagent in a single well
Sample plate 1	Buffer MB: 400 μL Isopropanol: 200 μL Post-conversion product: 220 μL Magbead solution: 20 μL
Washing plate 2	Wasing WB：500 μL
Desulfonate substrate 3	Buffer DB：200 μL
Washing plate 4	Wasing WB：500 μL
Wasing plate 5	Washing WB：200 μL
Elution plate 6	Elution Buffer：40-80 μL

3.4 Place the deep well plate with the sample into the instrument and run the program. After the program is finished, the eluted products are transferred to a new centrifuge tube for reserve (it is better to test immediately, if not immediately downstream test, please store the eluted products below -20 ℃).

Cautions

1. DNA modified by bisulfite is generally present in single strand or non-specific pairing form. When the purified genomic DNA OD260 is 1, it corresponds to a nucleic acid concentration of approximately 40μg/mL.
2. Due to the particularity of nucleic acid state after treatment, OD230 will appear abnormal phenomenon, which may lead to the instability of OD260/OD230 ratio. Experiments show 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 ddH2O is used, the ratio will be low because the pH and ion presence will affect the light absorption value, but does not indicate a low purity.
3. The converted liquid contains ammonium bisulfite, a low toxic compound. Do not touch skin and eyes during operation. If exposed, dry quickly and rinse with plenty of water. The product itself is free from fire and explosion risk. In case of fire, put out with water in time. After use, the waste liquid shall be stored in the waste liquid bottle and handed over to the third party for professional treatment.

Applicable instrument

1. Manual: Magnetic rack, recommended magnetic rack DynaMag™-2 (Cat.No. 12321D)
2. Automatic: Automatic extraction instrument, recommended to use Jiangsu Cowin Century Biotechnology Co., LTD. CWE960, CWE2100 products.

Sample requirements

1. This kit is suitable for extraction and purification of DNA solutions from feces, blood, tissues, and body fluids. The DNA input in a single conversion is about 100 pg-2 μg.
2. DNA after bisulfite modification is recommended to be tested immediately; otherwise, store it below -20°C for no more than 1 month.

Test method

1. Pre-use preparation :

1.1 For newly opened kit, add anhydrous ethanol to the Washing WB in advance according to the instructions on the reagent bottle label, and check whether anhydrous ethanol is added to the Washing WB before each use.

1.2 Take out the Conversion solution before use, balance it to room temperature and heat it (90℃ for 5 min) to fully dissolve and mix it (dissolution can be accelerated by vortex). After it is completely dissolved, stand at room temperature for several minutes away from light, and then open the cover for use after it has returned to room temperature.

Note: Reagent crystallization and resolution will not affect the effectiveness of the kit.

2. Manual DNA transformation:

2.1 Column method:

2.1.1 DNA samples should be equilibrated to room temperature in advance. Take 20 μL samples and add into a new PCR tube.

Note: The sample input should be between 100pg-2μg (different sample inputs can be selected according to the purpose of the experiment, 200 ng-500 ng is generally recommended), if less than 20 μL, add water to make up to 20 μL.

2.1.2 Add 200μL Conversion solution to the sample, the total volume is 220μL, mix and put into the PCR instrument to start the transformation.

The conversion procedure is as follows:

98°C	10 min
54°C	1 h

2.1.3 Add 200μL Buffer PS into the adsorption column loaded into the collection tube, centrifuge at 12000 rpm for 1 min, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

2.1.4 Add 600μL Buffer MB and 220μL transformed product to the adsorption column, upside down 3-5 times, and leave for 10 min at room temperature.

2.1.5 Centrifuge at 12,000 rpm for 1 min, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

2.1.6 Add 500 μL Washing WB to the adsorption column (check whether anhydrous ethanol has been added before use), and centrifuge at 12000 rpm for 1 min.

2.1.7 Add 200μL Buffer DB into the adsorption column, leave for 15-20 min at room temperature, centrifuge at 12000 rpm for 1 min, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

2.1.8 Add 500 μL Washing WB into the adsorption column and centrifuge at 12000 rpm for 1 min.

2.1.9 Add 200μL Washing WB into the adsorption column, centrifuge at 12000 rpm for 1 min, and discard the waste in the collection tube liquid, put the adsorption column back into the collection tube.

2.1.10 The collection tube with the adsorption column was centrifuged at 12,000 rpm for 2 min.

2.1.11 Transfer the adsorption column to a new 1.5mL centrifuge tube, open the cap, and let stand at room temperature for 3 min to volatilize the ethan cleanly.

2.1.12 Add 30-80 μL Elution Buffer to the middle part of the adsorption film (pay attention to the tip do not poke the adsorption film, change the tip when adding different samples), cap the tube tightly, and incubate at room temperature for 2 min. Centrifuge at 12000 rpm for 1 min collect the elution product and put it aside (it is best to test immediately. If the downstream test is not carried out immediately, please store the elution product below -20 ℃).
Note: Eluent must be preheated to 65℃.

2.2 Magbeads method

2.1.1 The DNA sample should be equilibrated to room temperature. Take 20 μL samples and add into a new PCR tube.
Note: The sample input should be between 100 pg-2 μg (different sample inputs can be selected according to the purpose of the experiment, 200 ng-500 ng is generally recommended), if less than 20 μL, add water to make up to 20 μL.

2.1.2 Add 200μL Conversion solution to the sample, the total volume is 220μL, mix and put into the PCR instrument to start the transformation.
The conversion procedure is as follows:

98°C	10 min
54°C	1 h

2.2.3 Take a new 1.5mL centrifugation tube, add 400μL Buffer MB, 200μL isopropanol, 220μL transformed product and 20μL magnetic bead solution in turn, shake and mix, and place on a constant temperature mixer at room temperature (10-30℃) at 1700 rpm for 10 min, and then centrifuge instantaneously.
Note: The magnetic beads must be thoroughly mixed before use. Magnetic bead settlement speed is fast, continuous add magnetic bead, must be mixed several times.

2.2.4 The centrifugal tube was placed on the magnetic stand for 30 seconds, and the waste liquid was absorbed and discarded.

2.2.5 Add 500μL Washing WB into the centrifuge tube. mix well by pipette or shaking , centrifuge instantaneously and place on magnetic rack for 30 s to absorb and aspirate the waste solution.

2.2.6 Add 200μL Buffer DB into the centrifuge tube, mix well by pipette or shaking, place on a constant temperature mixer, incubate at room temperature (10-30 ℃) at 1700 rpm for 15-20 min, centrifuge instantaneously, place on a magnetic rack for 30s, and then aspirate the waste solution.

2.2.7 Add 500μL Washing WB into the centrifuge tube, mix well by pipette or shaking, and then place it on magnetic rack for 30 s after instantaneous centrifugation, and then aspirate the waste solution.

2.2.8 Add 200μL Washing WB into the centrifuge tube, mix well by pipette or shaking, put it on a magnetic force rack for 30 s after a short centrifugation, and then aspirate the waste solution. The waste liquid is removed as clean as possible, and dry at room temperature for 5-10 min (the surface of the magnetic bead becomes matte and no crack is observed by visual observation).

2.2.9 Add 30-80 μL Eluent buffer into the centrifuge tube, mix it with pipetting or shaking, and place it on a constant temperature mixer for 10 min at 1700 rpm at 65 ℃. Centrifugation instantaneously, place it on the magnetic rack for 30s and transfer the eluted product to a new centrifuge tube for use (it is better to test it immediately. If the downstream test is not performed immediately, store the eluted product below -20 ℃).

3. Automatic conversion:

3.1 The DNA sample that needs to be methylated is pre-balanced to room temperature. Twenty μL samples were added to a new PCR tube.
Note: The sample input should be between 100 pg-2 μg (different sample inputs can be selected according to the purpose of the experiment, 200 ng-500 ng is generally recommended), if less than 20 μL, add water to make up to 20 μL.

3.2 Add 200μL Conversion solution to the sample, the total volume is 220μL, mix and put into the PCR instrument to start the transformation.
The conversion procedure is as follows: