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 1.5 mL 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°C).

Note: Eluent must be preheated to 65°C.

Version: 12/2023

Samples Preprocessing Kit for Methylation Test

Cat. No. : CW3078S (50 preps)

Storage Conditions: Adsorption column DF store at 2-8°C，Conversion solution store at -20±5°C in the dark，others store at 4-30°C.

Components

Component	CW3078S 50 preps
Conversion solution	8×1.5 mL
Buffer MB	30 mL
Buffer DB	10 mL
Washing WB	2×10 mL
Elution Buffer	4 mL
Buffer PS	10 mL
Adsorption column DF	50 Pcs
Collection Tube	50 Pcs

Introduction

The basic principle of this kit is that after DNA is treated with bisulfite, unmethylated cytosine can be converted into uracil, while methylated cytosine remains unchanged. And adopt the original high temperature treatment method, greatly shorten the time of methylation conversion, improve the conversion efficiency, the conversion efficiency can reach more than 99%.

This kit recovers DNA from bisulfite modified solution. The recovered DNA has high purity and good integrity, and can be directly used for sequencing and methylation PCR.

Storage conditions and expiration date

1. Transported at 4-37°C, and the transportation time is recommended not to exceed 7 days.
2. Adsorption column DF store at 2-8°C，Conversion solution store at -20±5°C in the dark，others store at 4-30°C. The validity period of the kit is 12 months.
3. The conversion solution is sensitive to light and easy to oxidize. Use it once after opening and freeze and thaw no 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.

Preparation and important notes before experiment

Take out the conversion solution in advance, balance it to room temperature and heat it (5 min at 90°C) to fully dissolve and mix it (can be accelerated by vortex dissolution). If it is not dissolved, the heating time can be extended appropriately. After it is completely dissolved, stand at room temperature for a few minutes away from light, and then open the cover when it returns to room temperature.

Note: Reagent crystallization and redissolution will not affect the effect of the kit.

Test method

1. Preparation before use:

1.1 For newly opened kits, pre-add anhydrous ethanol to Washing WB as instructed on the label of the reagent bottle, and check for anhydrous ethanol before each use.

1.2 The conversion solution should be taken out in advance, balanced to room temperature and heated (5 min at 90°C) to fully dissolve and mix (dissolution can be accelerated by vortex). If it is not dissolved, the heating time can be extended appropriately. After it is completely dissolved, stand at room temperature for a few minutes away from light, and then open the cover when it returns to room temperature.

Note: Reagent crystallization and redissolution will not affect the effect 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 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 200 μL Conversion solution to the sample, the total volume is 220 μL, 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 220 μL 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.