# DNA methylation kit

Project number: D665729

**Storage conditions:** room temperature.

**Product Content:**

| Component | D665729 10preps | D665729 50preps |
|---|---|---|
| CTConversionReagent | 10reactions | 50reactions |
| M-DissolvingBuffer | 10reactions | 50reactions |
| M-DilutionBuffer | 400 μl | 2ml |
| M-BufferPA | 15ml | 60ml |
| M-WashBuffer (concentrate) | 2ml | 10ml |
| M-ElutionBuffer | 1ml | 10ml |
| BufferPS | 5ml | 15ml |
| SpinColumnsDS withCollectionTubes | 10 | 50 |

**Product Description:**

The basic principle of this kit is that after DNA is treated with sodium bisulfite, the unmethylated cytosine can be transformed into uracil, while the methylated cytosine remains unchanged. And it adopts original high temperature treatment method, which greatly shortens the transformation time and improves the transformation efficiency, which can reach more than 99%. Meanwhile, the purified DNA can be recovered from the methylation-modified solution by a simple binding-washing-elution step using a silica matrix membrane-type purification column, and the recovered DNA is of high purity and integrity, which can be directly used in a variety of molecular biology experiments, such as sequencing, PCR detection of methylation, microarray analysis, ligations and transformations, digests, labeling, microinjections, PCR and in-vitro transcription, and other molecular biology experiments.

**Self-contained reagents:** anhydrous ethanol, 75% ethanol.

**Pre-experiment Preparation and Important Notes**

1. Methods of product formulation:

(1) 10-pack preparation method: CTConversionReagent is a solid mixture and must be prepared before first use. Add 2 ml of sterile water, 100 μl of

M-DissolvingBuffer and 300 μl of M-DilutionBuffer to the CTConversionReagent tube. Dissolve at 55° C and shake until all is dissolved. Store the CTConversionReagent solution at room temperature (20° C-30° C) away from light before use. Each tube of CTConversionReagent is designed for 10 DNA treatments. For better results, the configured CTConversionReagent should be used immediately, or if not, the CTConversionReagent solution can be stored at -20° C for 1 week before use. Before use, be sure to thaw the CTConversionReagent solution at room temperature and mix well by shaking or inverting for 2 minutes. CTConversionReagent is sensitive to light, so minimize exposure to light.

(2) Preparation method for 50 packages: CTConversionReagent and M-DissolvingBuffer are solid mixtures and must be prepared before the first use. Add 5ml of sterile water to M-DissolvingBuffer and shake to dissolve, after all the solids are dissolved, transfer all the solution in the M-DissolvingBuffer tube to the CTConversionReagent tube and add 5.5ml of sterile water at the same time. Add 1.5 ml of M-DissolvingBuffer to the CTConversionReagent tube. Dissolve at 55° C and shake until all is dissolved. Store the CTConversionReagent solution at room temperature (20° C-30° C) away from light before use. Each tube of CTConversionReagent is designed for 50 DNA treatments. For better results, CTConversionReagent should be used immediately after preparation, or if not used immediately, the CTConversionReagent solution can be stored at -20° C for 1 week before use. Before use, be sure to thaw the CTConversionReagent solution at room temperature and mix well by shaking or inverting for 2 minutes. CTConversionReagent is sensitive to light, so minimize exposure to light.

2. Anhydrous ethanol should be added to the M-WashBuffer according to the instructions on the label of the reagent bottle before first use.

## procedure

DNA ranged from 1 ng-4 μg per preparation, with an optimal amount of 500 ng-2 μg.

1. Take 20 μl of DNA sample and add it to the centrifuge tube (provided), if the sample amount is not enough, make up to 20 μl with water. 2. Add 2.2 μl of M-DilutionBuffer to the DNA sample and mix the sample.

3. 42° C water bath for 30 minutes.

4. To the sample obtained in the previous step, add 220 μl of prepared CTConversionReagent solution, mix well, and incubate in a constant temperature water bath at 80℃ for 60 minutes away from light.

5. Add 480 μl of M-BufferPA to the solution in the previous step and mix gently by turning up and down.

6. Column equilibration: Add 200 μl BufferPS to the adsorbent column (SpinColumnsDS) that has been loaded into the collection tube, centrifuge

at 12,000 rpm (~13,400 x g) for 2 minutes, pour off the waste liquid in the collection tube, and put the adsorbent column back into the collection tube.

7. Add all of the solution obtained in step 5 to the adsorption column (already in the collection tube), leave at room temperature for 2 minutes, centrifuge at 12,000 rpm for 1 minute, pour off the waste liquid from the collection tube and put the column back into the collection tube.

Note: The maximum capacity of the adsorption column is 750μl, if the sample volume is larger than 750μl, it can be added in batches.

8. Add 500 μl of M-BufferPA to the adsorbent column and centrifuge at 12,000 rpm for 1 min, pour off the waste liquid in the collection tube, and put the adsorbent column back into the collection tube.

9. Add 650 μl of M-WashBuffer to the adsorbent column (check that anhydrous ethanol has been added before use), centrifuge the column at 12,000 rpm for 1 minute, pour off the waste liquid in the collection tube, and place the column back into the collection tube.

10. Centrifuge the column at 12,000 rpm for 2 minutes, pour off the waste solution and leave the column at room temperature for a few minutes to dry thoroughly.

Note: The purpose of this step is to remove residual ethanol from the adsorbent column, which can interfere with subsequent enzymatic reactions (digestion, PCR, etc.). 11. Place the column in a new centrifuge tube (supplied) and add 20 μl of M-Elution to the center of the adsorbent membrane.

Buffer (pH 8.5) and leave at room temperature for 2 min. centrifuge at 12,000 rpm for 1 min to collect the DNA solution.12. Add 2.2 μl of M-DilutionBuffer to the collected 20 μl of DNA and leave for 30 min at room temperature.

13. Add 500 μl of pre-cooled anhydrous ethanol to the solution and mix upside down, and place the solution at -20° C for 30 minutes to precipitate (overnight precipitation is better).

14. Centrifuge at 12,000 rpm for 15 minutes and gently pour off the supernatant.

15. Add 75% ethanol, centrifuge at 12,000 rpm for 1 minute, pour off the supernatant, wait for the ethanol to evaporate at room temperature, then add 20 μl of M-ElutionBuffer to solubilize the DNA, and store the DNA at -20℃. The DNA collected in this step can be used for subsequent related experiments.