

Urine DNA Methylation Pretreatment Kit

【REF】

UC-MS02

【Package Size】

24 tests/kit, 48 tests/kit, 96 tests/kit

【Intended Use】

The urine DNA methylation pretreatment kit is intended for rapid and reliable DNA bisulfite conversion, extraction, enrichment, and purification for methylation analysis.

【Principle of Procedure】

Utilizes bisulfite treatment to convert unmethylated cytosine (C) into uracil (U).

【Reagents Provided】

Component	24 tests	48 tests	96 tests
Lysis solution	1×8mL	2×8mL	4×8mL
Conversion solution	1×3.5mL	2×3.5mL	4×3.5mL
Binding solution	1×15mL	2×15mL	4×15mL
Desulfonation solution	1×6mL	2×6mL	4×6mL
Washing solution	1×30mL	2×30mL	4×30mL
Elution solution	1×1mL	2×1mL	4×1mL
Magnetic Beads B	1×0.3mL	2×0.3mL	4×0.3mL

【Storage and Handling】

Store at 2-30°C. Do not freeze.

Do not use product after the expiration date.

【Specimen Collection and Preparation for Analysis】

Sample Type:

- 1) Fresh urine sample (first morning urine is preferred)
- 2) Urine collected by Urine collection kit (US-MS01).

【Instructions For Use】

1. Collect approximately 45 mL of urine in a urine preservative tube. If a frozen sample, it needs to be thawed for later use.
2. Take 5-10mL of urine sample and transfer it to a 15mL centrifuge tube. Centrifuge at 3500RPM for 10 minutes at

room temperature. Pour out the supernatant and obtain the precipitated cell pellets.

3. Add 150 µL of **Lysis solution** to the cell pellets and vortex thoroughly, then incubate at room temperature for 10 minutes. Take 40 µL of lysates (containing cell DNA) for the following sulfite conversion.
4. Prepare magnetic racks, 1.5mL centrifuge tubes, and 0.2mL PCR reaction tubes.
5. Prepare the bisulfite conversion reaction mix in a 0.2 ml PCR tube as follows:

Component	Quantity (µL)
DNA samples	10-40µL
Conversion solution	130µL
Total volume	140-170µL

6. Set up PCR instrument for DNA bisulfite conversion, 95°C-98°C for 8 minutes, 54°C for 30-60 minutes, and 4°C.
7. After the bisulfite treatment is completed, transfer the reaction system to a 1.5mL centrifuge tube.
8. Add 600 µL of **Binding solution** and 10 µL of **Magnetic Beads B**, vortex and incubate at room temperature for 5 minutes.
9. Place the centrifuge tube on a magnetic rack for 5 minutes, discard the supernatant after the magnetic beads are completely adsorbed.
10. Add 400 µL of **Washing solution** to the centrifuge tube and vortex tube for 5 sec.
11. Place the centrifuge tube on a magnetic rack for 1-3 minutes. Discard the supernatant after the magnetic beads are completely adsorbed.
12. Add 200 µL of **Desulfonation solution** to a centrifuge tube, mix well, and incubate at room temperature for 10-20 minutes.
13. Place the centrifuge tube on a magnetic rack for 1-3 minutes, then discard the supernatant.
14. Add 400 µL of **Washing solution** to a centrifuge tube and mix well.
15. Place the centrifuge tube on a magnetic rack for 1-3 minutes, then discard the supernatant.

16. Add 400 μ L of **Washing solution** to a centrifuge tube and mix well.
17. Place the centrifuge tube on a magnetic rack for 1-3 minutes, remove the supernatant, dry the magnetic beads until there is no liquid residue.
18. Add 25 μ L of **Elution solution**, resuspend beads, and let sit at room temperature for 5 minutes.
19. Place the centrifuge tube on a magnetic rack for 1 minute. Collect cleared supernatant (containing converted DNA). The DNA concentration detected by the QuBit instrument should not be less than 0.1ng/ μ L.
20. Converted DNA directly used for downstream experiments or stored at -20 °C.

【Limitations】

1. This product is only for bisulfite conversion, extraction, enrichment, and purification of nucleic acids.
2. The processed product is suitable for clinical gene methylation analysis.

【Warnings and Precautions】

1. For research use only.
2. Do not use if the components in kit show color change or precipitate.
3. Do not use if the reagent kit components exceed the expiry date.
4. Avoid microbial contamination.
5. Take safety precautions when handling or using any biological specimen or reagent.

【Contact Information】

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