

EpiArt Magnetic DNA Methylation Bisulfite Kit

EM103



Instruction for Use

Version 26.1

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For Research Use Only. Not for use in diagnostic procedures.

01/Product Description

EpiArt Magnetic DNA Methylation Bisulfite Kit is specifically designed for efficient DNA conversion. It integrates DNA denaturation and bisulfite conversion into a single step, reducing the total processing time to approximately 100 min. Following bisulfite treatment, the converted DNA binds to magnetic beads, enabling efficient desulfonation and purification. The converted product is fully compatible with a variety of downstream applications, including PCR, qPCR, restriction enzyme analysis, and sequencing. With an optimized reaction system, this kit supports 100 pg - 2 µg of input DNA and achieves ≥99.5% conversion efficiency of unmethylated cytosines. It is also compatible with automated workstations and suitable for studies of physiological and pathological processes, such as genomic imprinting, embryonic development, chromosomal gene silencing, and cell cycle regulation.

02/Components

Components	EM103-01 (50 rxns)	EM103-02 (200 rxns)
■ CT Conversion Powder	5 × 10 rxns	20 × 10 rxns
■ CT Conversion Diluent	1.5 ml	6 ml
■ CT Conversion Buffer	250 µl	1 ml
■ E-Binding Beads	500 µl	2 × 1 ml
■ E-Binding Buffer	30 ml	2 × 60 ml
■ E-Wash Buffer	20 ml	2 × 30 ml
■ E-Desulphonation Buffer	10 ml	40 ml
□ E-Elution Buffer	2 × 1 ml	5 × 1 ml

- ▲ CT Conversion Powder: Powder for bisulfite conversion reaction.
- CT Conversion Diluent: Dilution Buffer for CT Conversion Powder.
- CT Conversion Buffer: Dissolution Buffer for CT Conversion Powder.
- E-Binding Beads: Magnetic beads for binding converted DNA.
- E-Binding Buffer: Buffer for E-Binding Beads.
- E-Wash Buffer: Wash buffer for removing salts from DNA.
- E-Desulphonation Buffer: Buffer for desulphonation.
- E-Elution Buffer: Buffer for DNA elution.

03/Storage

Store at 15 ~ 25°C and ship at room temperature.

The reconstituted CT Conversion Mix should be protected from light. Prepare it immediately before use. If needed, store it for up to 24 h at room temperature, 1 week at 0 ~ 4°C, or 1 month at -30 ~ -15°C.

04/Applications

The kit is compatible with DNA templates extracted from cells or tissues of animals, plants, and microorganisms, as well as cell-free DNA (cfDNA).

DNA input: 100 pg - 2 µg; 10 ng - 1 µg for optimal performance.

05/Self-prepared Materials

Absolute ethanol, Nuclease-free ddH₂O, 1.5 ml Nuclease-free tubes, Nuclease-free PCR tubes, Nuclease-free pipette tips, vortex mixer, magnetic rack, PCR instrument, and mini centrifuge.

06/Notes

1. To ensure reliable bisulfite conversion efficiency, unmethylated lambda DNA is recommended as a negative control and should be spiked in at 1% (w/w) mass ratio prior to conversion. If fragmentation is required, the control DNA should be added before fragmentation. Conversion efficiency can be evaluated by analyzing the C-to-T conversion rate of lambda DNA in the sequencing data.
2. A small amount of precipitation may occur in CT Conversion Mix and does not affect performance. Prepare the mix immediately before use. Otherwise, it can be stored for up to 24 h at room temperature, 1 week at 0 ~ 4°C, or 1 month at -30 ~ -15°C. Before use, resuspend thoroughly, heat at 37°C for 10 min, equilibrate to room temperature, and mix well by vortexing. The mix should be protected from light.
3. Store E-Binding Beads at room temperature (15 ~ 25°C) or at 0 ~ 4°C. Before each use, equilibrate to room temperature and mix thoroughly by vortexing. Do not store the beads at -30 ~ -15°C.
4. Before use, add absolute ethanol to the E-Wash Buffer (80 ml per bottle for Vazyme #EM103-01 and 120 ml per bottle for Vazyme #EM103-02). Cap the bottle tightly to prevent evaporation.
5. Bead sedimentation may occur with ≥1 µg DNA input; this does not affect the results.
6. Cap the CT Conversion Buffer and CT Conversion Mix tightly after use.
7. Cap E-Desulphonation Buffer tightly to prevent evaporation.
8. Store the bisulfite-converted DNA at -30 ~ -15°C. For long term storage, store at -85 ~ -65°C and avoid repeated freeze-thaw cycles.
9. The converted product is single-stranded DNA and is recommended to be quantified using the Equalbit ssDNA Assay Kit (Vazyme #EQ311).

07/Mechanism & Workflow

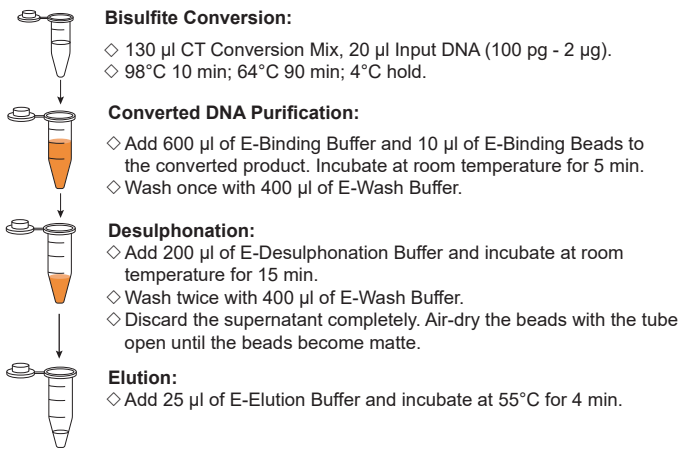


Fig 1. Workflow of EpiArt Magnetic DNA Methylation Bisulfite Kit

08/Experiment Process

08-1/Reagent Preparation

- Before use, add absolute ethanol to the E-Wash Buffer (80 ml per bottle for Vazyme #EM103-01 and 120 ml per bottle for Vazyme #EM103-02). Cap the bottle tightly to prevent evaporation.
- CT Conversion Mix Preparation: Add 900 µl of Nuclease-free ddH₂O, 300 µl of CT Conversion Diluent, and 50 µl of CT Conversion Buffer to the CT Conversion Powder, and vortex at room temperature for 5 - 10 min until fully dissolved. Each prepared CT Conversion Mix tube is sufficient for 10 reactions.
 - ▲ A small amount of precipitation may occur in CT Conversion Mix and does not affect performance. Prepare the mix immediately before use. Otherwise, it can be stored for up to 24 h at room temperature, 1 week at 0 ~ 4°C, or 1 month at -30 ~ -15°C. Before use, resuspend thoroughly, heat at 37°C for 10 min, equilibrate to room temperature, and mix well by vortexing. The mix should be protected from light.
 - ▲ Cap the CT Conversion Buffer and CT Conversion Mix tightly after use.

08-2/Bisulfite Conversion

- Equilibrate CT Conversion Mix to room temperature and mix well by vortexing. Prepare the following reaction in a Nuclease-free PCR tube:

Components	Volume
CT Conversion Mix	130 µl
Input DNA (100 pg - 2 µg)	X µl
Nuclease-free ddH ₂ O	To 150 µl

- ▲ For library preparation, the input DNA is recommended to be fragmented to ~600 bp before conversion.

- Mix well by vortexing or pipetting, and collect the reaction mixture at the bottom of the tube.
- Place the tube in the PCR instrument and run the following program:

Temperature	Time
Heated lid 105°C	On
98°C	10 min
64°C	90 min
4°C	Hold (<20 h)

▲ Set the PCR instrument with a heating volume of $\geq 100 \mu\text{l}$.

08-3/Purification

- Add 600 μl of E-Binding Buffer, 10 μl of E-Binding Beads, and the bisulfite-converted DNA from the previous step to a 1.5 ml Nuclease-free centrifuge tube. Mix well by pipetting for 6 - 8 times or low-speed vortexing for 30 sec, and incubate at room temperature for 5 min.

▲ E-Binding Beads stored at 0 ~ 4°C should be equilibrated to room temperature before each use.
Mix the beads thoroughly by vortexing before each use.

- Briefly centrifuge the tube and place it on a magnetic rack until the supernatant is clear (~3 min), carefully discard the supernatant.
- Add 400 μl of E-Wash Buffer (with the specified volume of absolute ethanol added) to the tube and mix well by pipetting for 6 - 8 times or low-speed vortexing for 30 sec. Briefly centrifuge the tube and place it on a magnetic rack until the supernatant is clear (~3 min), then carefully discard the supernatant.
- Add 200 μl of E-Desulphonation Buffer to the tube and mix well by pipetting for 6 - 8 times or low-speed vortexing for 30 sec. Incubate at room temperature for 15 min. Briefly centrifuge the tube and place it on a magnetic rack until the supernatant is clear (~3 min), then carefully discard the supernatant.

▲ Do not exceed 25 min in E-Desulphonation Buffer, including bead resuspension, incubation, and supernatant removal.

- Add 400 μl of E-Wash Buffer (with the specified volume of absolute ethanol added) to the tube and mix well by pipetting for 6 - 8 times or low-speed vortexing for 30 sec. Briefly centrifuge the tube and place it on a magnetic rack until the supernatant is clear (~3 min), then carefully discard the supernatant.
- Repeat Step 5, and discard the supernatant. Wash twice in total.
- Dry at 55°C for 5 - 15 min or at room temperature for 20 - 30 min until the beads become matte.

▲ Please dry the beads thoroughly to avoid affecting downstream experiments; fully dried beads will change from dark brown to brown.

8. Add 25 µl of E-Elution Buffer to the tube and resuspend the beads by pipetting for 6 - 8 times or low-speed vortexing for 30 sec. Incubate at 55°C for 4 min. Briefly centrifuge the tube and place it on a magnetic rack until the supernatant is clear (~3 min), then transfer the supernatant to a new tube.
9. Store the converted DNA at -30 ~ -15°C. For long term storage, store at -85 ~ -65°C. Avoid repeated freeze-thaw cycles.

09/FAQ & Troubleshooting

Question	Solutions
About Input DNA	DNA dissolved in Nuclease-free ddH ₂ O or TE buffer; input amount of 100 pg - 2 µg in 20 µl; A260/A280 ratio of 1.6 - 2.0.
Can the bead volume be increased to improve DNA recovery?	Yes; however, do not exceed 15 µl. Larger bead volumes may cause bead aspiration. If this occurs, place the tube on a magnetic rack and recover the supernatant.
How to simplify the procedure when processing multiple samples?	During the purification of the converted DNA (Step 3 of 08-3/ Purification), it is recommended to resuspend the beads in 200 µl of E-Wash Buffer and transfer the entire suspension to an 8-strip tube to minimize sample loss. After desulphonation, the E-Wash Buffer volume can also be adjusted to 200 µl. Subsequent steps can be performed using a multichannel pipette.

Question	Possible Causes	Solutions
Low DNA recovery	Impurities in the input DNA	Ensure a DNA A260/A280 ratio of 1.6 - 2.0.
	Insufficient ethanol in E-Wash Buffer	Add the specified volume of absolute ethanol and cap the bottle immediately.
	Insufficient organic solvent in E-Desulphonation Buffer	Cap the bottle immediately.
	Prolonged desulphonation time (>25 min)	Ensure the desulphonation is completed within 25 min.
Low conversion rate	Expired CT Conversion Mix	Properly dissolve and store the CT Conversion Mix, and use it within its shelf life.
	Improper reaction temperature or time	Set the reaction temperature and time according to the instructions.
	High GC content or out-of-range input DNA	Extend the conversion from 90 min at 64°C to 2.5 h at 64°C.



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