EpiArt DNA Enzymatic Methylation Kit

EM301

Instruction for Use

Version 23.1

Vazyme

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01/Product Description

EpiArt DNA Enzymatic Methylation Kit is an enzyme-based DNA methylation kit. Compared with the sulfite conversion method, this product has the advantages of less DNA damage, better sequencing data uniformity, and higher detection sensitivity of CG sites. It can be combined with the next-generation sequencing (NGS) technology to enable highly accurate identification of DNA methylation sites at single-base resolution. The conversion product obtained with this kit can be used for downstream applications such as conventional polymerase chain reaction (PCR), fluorescent quantitative PCR (qPCR), and NGS library preparation.

02/Components

	Components	EM301-01 (24 rxns)	EM301-02 (96 rxns)
■ (red)	Oxidation Reaction Buffer	120 μl	480 μl
■ (red)	Oxidation Reaction S	4 × 24 μl	4 × 96 μl
■ (red)	Oxidation Reagent	24 μl	96 μl
■ (red)	Oxidation Enzyme 1	240 μl	960 μl
■ (red)	Oxidation Enzyme 2	24 μl	96 μl
■ (green)	Active Reagent	24 μl	4 × 24 μl
□ (white)	Stop Reagent	24 μl	96 μl
■ (yellow)	DEA Reaction Buffer	240 μl	960 μl
■ (yellow)	BSA	24 μl	96 μl
■ (yellow)	DEA Enzyme Mix	24 μl	96 μl
■ (blue)	Dilution Buffer	1 ml	4 × 1 ml

03/Storage

Store at -30 ~ -15°C and transport at ≤0°C.

04/Applications

1. Compatible template types:
    - ◆ DNA (genomic DNA) extracted from tissues or cells of animals or plants.
    - ◆ Cell-free DNA (cfDNA).
2. Requirement of template quality:
   It is recommended to dissolve the sample in ddH_2O (without EDTA).
3. Compatible template amount: 10 - 200 ng.

05/Self-prepared Materials

1. DNA quantification kits:
   Equalbit 1 × dsDNA HS Assay Kit (Vazyme #EQ121).
   Qubit ssDNA Assay Kit (Invitrogen #Q10212).

2. Magnetic beads for purification:
   VAHTS DNA Clean Beads (Vazyme #N411).

3. Library preparation kits:
   Single-stranded DNA (ssDNA) library preparation kit: EpiArt DNA Methylation Library Kit for Illumina V3 (Vazyme #NE103).
   Double-stranded DNA (dsDNA) library preparation kit: VAHTS Universal DNA Library Prep Kit for Illumina V4 (Vazyme #ND610) combined with the amplification module Phanta Uc Super-Fidelity DNA Polymerase for Library Amplification (Vazyme #P507).

4. Other materials:
   Absolute ethanol, 1 M NaOH, Nuclease-free ddH$_2$O, magnetic rack, PCR instrument, low-binding EP tubes, Nuclease-free PCR tubes, etc.

06/Notes

For research use only. Not for use in diagnostic procedures.

1. For the first use, please mark the unsealing time on the "Oxidation Reaction S" tube, and use it up within three months.

2. Please dilute the Active Reagent (1:1000 dilution) before use. The diluted reagent is only used for the current experiment. Please discard in time after use, do not reuse.

3. Prepare 1 M NaOH solution. NaOH is susceptible to inactivation due to reaction with CO_2 in the air, so it should be sealed for storage after preparation. The following methods are recommended for storing and using NaOH:
   a. Aliquot the prepared 1 M NaOH to 1.5 ml EP tubes for long-term storage at -20°C. Use one tube per experiment. Please discard in time after use, do not reuse.
   b. 0.1 M NaOH is only used for the current experiment. Please discard in time after use, do not reuse.

4. Cap reagent vials tightly after use to avoid reagent oxidation.

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07/Mechanism & Workflow

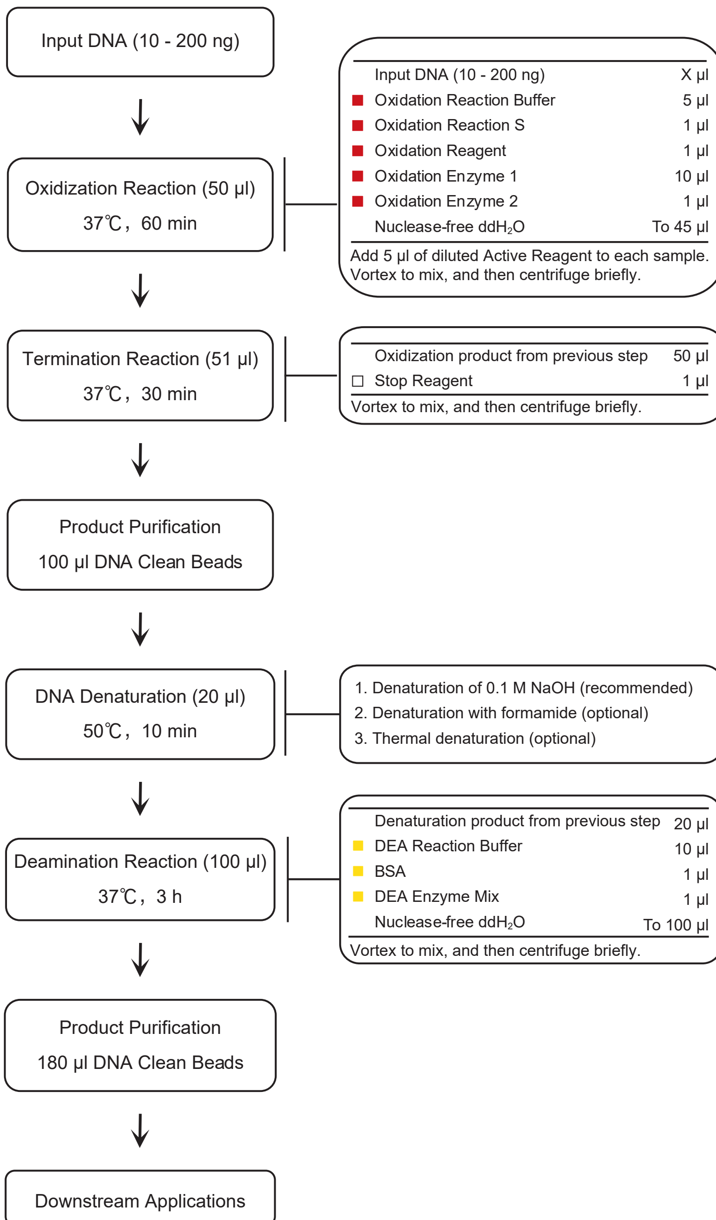

Fig 1. Enzymatic Methylation Workflow of EpiArt DNA Enzymatic Methylation Kit

08/Experiment Process

08-1/DNA Template Preparation

Template processing according to different downstream applications:

1. For conventional PCR and qPCR: directly use templates for experiments without fragmentation.
2. For NGS library preparation:
   a. Genomic DNA: Fragment the template to appropriate sizes in an ultrasonic homogenizer.
   b. cfDNA: directly use templates for experiments without fragmentation.

08-2/Oxidization Reaction

1. Take out Oxidation Reaction Buffer, Oxidation Reaction S, Oxidation Reagent, Oxidation Enzyme 1, Oxidation Enzyme 2, and Active Reagent, then thaw and mix well. Briefly centrifuge to the bottom of the tube and place it on ice for later use.
2. Add 1 μl of Active Reagent to 999 μl of Nuclease-free ddH_2O and mix well. Briefly centrifuge to the bottom of the tube and place it on ice for later use. Perform all the following steps on ice.
3. Prepare the reaction system in a Nuclease-free PCR tube according to the following table:
   ▲ For the first use, please mark the unsealing time on the "Oxidation Reaction S" tube, and use it up within three months.

Components	Volume	
Input DNA (10 - 200 ng)	X μl	
Oxidation Reaction Buffer	5 μl	■
Oxidation Reaction S*	1 μl	■
Oxidation Reagent	1 μl	■
Oxidation Enzyme 1	10 μl	■
Oxidation Enzyme 2	1 μl	■
Nuclease-free ddH_2O	To 45 μl	

4. Add 5 μl of diluted Active Reagent to each sample. Vortex to mix, and then centrifuge briefly to collect the reaction mix to the bottom of the tube.
   ▲ Make sure that Active Reagent has been diluted by 1,000 folds. Use the diluted Active Reagent for the current experiment only. Do not reuse.
5. Place the PCR tube in a PCR instrument and run the program: 37°C 60 min, 4°C hold.

08-3/Termination Reaction

This step allows digestion of the remaining enzyme after 08-2/Oxidization Reaction to terminate oxidization reaction.

1. Take out Stop Reagent and mix well. Briefly centrifuge to the bottom of the tube and place it on ice for later use.

2. Add 1 μl of Stop Reagent into the oxidization product from the previous step. Vortex to mix, and then centrifuge briefly to collect the reaction mix to the bottom of the tube.

3. Place the PCR tube in the PCR instrument and run the program: 37°C 30 min, 4°C hold.

4. Upon completion of the reaction, purify the reaction product using VAHTS DNA Clean Beads:

   a. Mix VAHTS DNA Clean Beads thoroughly by vortexing after equilibrating the magnetic beads to room temperature.

   b. Add 100 μl of the beads to the above reaction system, and mix thoroughly by gently pipetting up and down 10 times.

   c. Incubate the mixture at room temperature for 5 min to allow the DNA to bind to the beads.

   d. Centrifuge the PCR tube briefly. Place it on a magnetic rack until the solution is clear (about 3 - 5 min). Then, carefully remove the supernatant.

   e. Keep the PCR tube on the magnetic rack. Add 200 μl of freshly prepared 80% ethanol to rinse the magnetic beads. Incubate at room temperature for 30 sec, and then carefully remove the supernatant.

      ▲ Do not disturb the beads when adding 80% ethanol.

   f. Repeat Step e once.

   g. Keep the PCR tube on the magnetic rack. Uncap the tube and air-dry the beads at room temperature for about 3 - 5 min.

      ▲ Use a 10 μl pipette to remove all the residual liquid to shorten the drying time.

      ▲ Avoid over drying the beads (appear cracked), which may result in low recovery.

      ▲ The relative humidity varies across regions, and so does the drying time of magnetic beads. The magnetic beads should be air-dried to the degree that the surface changes from glossy brown to matte brown.

   h. Remove the PCR tube from the magnetic rack and add 18 μl of Nuclease-free ddH$_2$O. Mix well by gently pipetting up and down, and then incubate at room temperature for 2 min.

   i. Place the tube on a magnetic rack until the solution is clear (about 5 min). Carefully transfer 16 μl of the supernatant to a new Nuclease-free PCR tube.

      ▲The sample of this step can be stored in a -20°C refrigerator overnight.

08-4/DNA Denaturation

This step allows the denaturation of dsDNA to ssDNA to enable the normal deamination reaction. You may choose one of the following three DNA denaturation methods:

◆ Denaturation with 0.1 M NaOH.

◆ Denaturation with formamide.

◆ Thermal denaturation.

1. Denaturation with 0.1 M NaOH (recommended)
   a. Before starting, dilute 1 M NaOH by 10 folds to 0.1 M NaOH.
      ▲Use 0.1 M NaOH solution for the current experiment only. Do not reuse.

   b. Place the purification product from 08-3/Termination Reaction on ice. Add 4 μl of 0.1 M NaOH to each sample. Vortex to mix, and then centrifuge briefly to collect the reaction mix to the bottom of the tube.

   c. Place the PCR tube in the PCR instrument (with heated lid), and run the program: 50°C 10 min, 4°C hold.
      ▲Upon completion of the reaction, immediately place the reaction product on ice and let it stand for 2 min to avoid ssDNA renaturation, which will affect subsequent reactions.

2. Denaturation with formamide (optional)
   a. Place the purification product from 08-3/Terminatio Reaction on ice. Add 4 μl of formamide to each sample, vortex to mix, and centrifuge briefly to collect the reaction mix to the bottom of the tube.

   b. Place the PCR tube in the PCR instrument (with heated lid), and run the program: 85°C 10 min, 4°C hold.
      ▲Upon completion of the reaction, immediately place the reaction product on ice. Let it stand for 2 min to avoid renaturation of ssDNA to dsDNA, which will affect subsequent reactions.

3. Thermal denaturation (optional)
   a. Place the purification product from 08-3/Terminatio Reaction on ice. Add 4 μl of Nuclease-free ddH_2O to each sample. Vortex to mix, and then centrifuge briefly to collect the reaction mix to the bottom of the tube.

   b. Place the PCR tube in the PCR instrument with the heated lid activated, and run the program: 95°C 2 min, 4°C hold.
      ▲Upon completion of the reaction, immediately place the reaction product on ice. Let it stand for 2 min to avoid renaturation of ssDNA to dsDNA, which will affect subsequent reactions.

08-5/Deamination Reaction

This step allows deamination of the denaturation product from the previous step.

1. Take out DEA Reaction Buffer, BSA, and DEA Enzyme Mix, then thaw and mix well. Briefly centrifuge to the bottom of the tube and place it on ice for later use. Perform all the following steps on ice.

2. Prepare the following reaction system in a PCR tube:

Components	Volume
Denaturation product from previous step	20 μl
DEA Reaction Buffer	10 μl
BSA	1 μl
DEA Enzyme Mix	1 μl
Nuclease-free ddH_2O	To 100 μl

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3. Vortex to mix, and then centrifuge briefly to collect the reaction mix to the bottom of the tube.

4. Place the PCR tube in the PCR instrument and run the program: 37°C 3 h, 4°C hold.

   ▲The sample of this step can be stored in a -20°C refrigerator overnight.

5. Upon completion of the reaction, purify the reaction product using VAHTS DNA Clean Beads:

   a. Mix VAHTS DNA Clean Beads thoroughly by vortexing after equilibrating the magnetic beads to room temperature.

   b. Add 180 μl of the beads to the above reaction system, and mix the solution thoroughly by gently pipetting up and down 10 times.

   c. Incubate the mixture at room temperature for 10 min to allow the DNA to bind to the beads.

   d. Centrifuge the PCR tube briefly. Place it on a magnetic rack until the solution is clear (about 10 min). Then, carefully remove the supernatant.

   e. Keep the PCR tube on the magnetic rack. Add 200 μl of freshly prepared 80% ethanol to rinse the magnetic beads, incubate at room temperature for 30 sec, and carefully remove the supernatant.

      ▲ Do not disturb the beads when adding 80% ethanol.

   f. Repeat Step e once.

   g. Keep the PCR tube on the magnetic rack. Uncap the tube and air-dry the beads at room temperature for about 3 - 5 min.

      ▲ Use a 10 μl pipette to remove all the residual liquid to shorten the drying time.

      ▲ Avoid over drying the beads (appear cracked), which may result in low recovery.

      ▲ The relative humidity varies across regions, and so does the drying time of magnetic beads. The magnetic beads should be air-dried to the degree that the surface changes from glossy brown to matte brown.

   h. Remove the PCR tube from the magnetic rack and add 23 μl of Dilution Buffer. Mix well by gently pipetting up and down, and then incubate at room temperature for 2 min.

   i. Place the tube on a magnetic rack until the solution is clear (about 5 min), and then carefully transfer 20 μl of the supernatant to a new Nuclease-free PCR tube.

      ▲ The product of this step can be stored at -20°C for 2 days. Please perform the downstream experiments in time.

      ▲ It is recommended to use EpiArt DNA Methylation Library Kit for Illumina V3 (Vazyme #NE103) for downstream library preparation. For library preparation, the number of amplification cycles can be set according to the input amount of the initial template for enzymatic conversion.

09/FAQ & Troubleshooting

Question	Reasons	Solutions
Discoloration of some reagents after long-term storage	Oxidation Reaction Buffer, Oxidation Reaction S, and Active Reagent may be oxidized and become light yellow after long-term storage or repeated opening.	The performance of these reagents remains unaltered, permitting continued use.
Too low measurement of ssDNA at a low template input amount	For the excessively low concentration of ssDNA, the measured value may be inaccurate, which will not affect the subsequent library preparation.	Set the number of PCR amplification cycles directly according to the input amount of initial templates.
Low deamination efficiency	1. NaOH may have reacted with CO_2 in the air and become inactivated due to improper storage. 2. The product may not have been placed on ice immediately upon denaturation, causing ssDNA renaturation.	1. Store 1 M NaOH according to 06/Notes upon preparation. 2. Place the product on ice immediately upon completion of the reactions in 08-4/DNA Denaturation. Do not wait for the temperature to drop.
Degradation of deamination product	1. The product may have been stored at improper temperatures and degraded. 2. Nuclease residues may be present in the environment and degrade the product.	1. Promptly use the deamination product for downstream experiments. 2. Regularly clean the experimental environment to remove nuclease residues.

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