EpiArt Magnetic DNA Methylation Bisulfite Kit

NB-54-0375-1

NB-54-0375-2

Contents

Epiart Magnetic DNA Methylation Bisulfite Kit

#Cat: NB-54-0375-1 Size: 50rxns

#Cat: NB-54-0375-2 Size: 200rxns

1/Product Description

DNA methylation is closely related to gene expression and function. It plays a key role in a range of physiological and pathological processes, including genomic imprinting, embryonic development, chromosome gene silencing, and cell cycle regulation. EpiArt Magnetic DNA Methylation Bisulfite Kit is compatible with 100 pg - 2 µg of input DNA and enables ≥99.5% conversion efficiency of non-methylated cytosines. This kit integrates DNA denaturation and bisulfite conversion into one step, and shortens the conversion reaction time to 100 min. Desulphonation and purification of the converted DNA are performed while bound to beads. The bisulfite-con- verted DNA can be used for downstream applications such as PCR, qPCR, endonuclease digestion, and NGS. The kit is compatible with automated high-throughput systems.

2/Components

	Components	NB-54-0375-01 (50 rxns)	NB-54-0375-02 (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: CT conversion reagent.
CT Conversion Diluent: Dilutes CT Conversion Powder.
CT Conversion Buffer: Dissolves CT Conversion Powder.
E-Binding Beads: Bind to converted DNA.
E-Binding Buffer: Provide a buffer environment for DNA-magnetic bead binding.
E-Wash Buffer: Remove salt ions from DNA.
E-Desulphonation Buffer: Remove sulphonic acid groups.
E-Elution Buffer: Elute DNA.

3/Storage

Store at 15 ~ 25°C and transport at room temperature.
Resuspended CT Conversion Mix should be stored away from light. For best results, the CT Conversion Mix Reagent is in a ready-to-use liquid format. Unused portions may be stored at room temperature (15 ~ 25°C) for 24 h, 0 ~ 4°C for 1 week, or -30 ~ -15°C for 1 month.

4/Applications

The kit is compatible with DNA templates from various sources: DNA (genomic DNA) extracted from tissues or cells of animals or plants; cfDNA (cell-free DNA). Input amount: 100 pg - 2 μg (use 10 ng - 1 μg for optimal results).

5/Self-prepared Materials

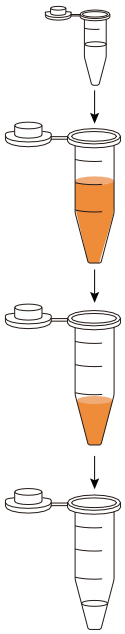
Ethanol absolute; Nuclease-free ddH_2O; 1.5 ml Nuclease-free centrifuge tubes; Nuclease-free PCR tubes; Nuclease-free pipette tips; vortex mixer; magnetic rack; PCR instrument; microcentrifuge.

6/Notes

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

1. Small amounts of precipitate may form in the CT Conversion Mix, which is normal and does not affect the experiment. Prepare the ready-to-use CT Conversion Mix before use. Unused portions may be stored at room temperature (15 ~ 25°C) for 24 h, 0 ~ 4°C for 1 week, or -30 ~ -15°C for 1 month. These stored reagents should be fully reconstituted before use. It is recommended that the reagents be heated at 37°C for 10 min, equilibrated to room temperature and vortexed. Keep CT Conversion Mix protected from light.
2. Store E-Binding Beads at room temperature (15 ~ 25°C) or 0 ~ 4°C. Equilibrate to room temperature and mix thoroughly by vortexing before each use. Do not freeze E-Binding Beads (-30 ~ -15°C).
3. Add the specified volume of ethanol absolute to E-Wash Buffer before the first use (NB-54-0375-01: 80 ml per bottle; NB-54-0375-02: 120 ml per bottle). Tightly cap the bottles to prevent ethanol evaporation.
4. When the DNA input amount is ≥1 μg, the beads tend to settle. This is normal and does not affect experiment results.
5. Tightly cap the CT Conversion Buffer and CT Conversion Mix after use.
6. E-Desulphonation Buffer contains volatile organic solvents. Cap tightly to prevent evaporation.
7. Store the bisulfite-converted DNA at -30 ~ -15°C. For long term storage, store at -85 ~ -65°C. Avoid repeated freeze-thaw cycles.

7/ Mechanism & Workflow



Bisulfite conversion:

- 130 μl of CT Conversion Mix, 20 μl of input DNA (100 pg - 2 μg)
- 98°C, 10 min; 64°C, 90 min; 4°C hold

Purification:

- Add 600 μl of E-Binding Buffer and 10 μl of E-Binding Beads to bisulfite-converted DNA; incubate at room temperature for 5 min
- Wash once with 400 μl of E-Wash Buffer
- Desulphonation: Add 200 μl of E-Desulphonation Buffer and incubate at room temperature (15 ~ 25°C) for 15 min
- Wash twice with 400 μl of E-Wash Buffer
- Discard the supernatant and air dry until the beads appear matte
- Elution: Add 25 μl of E-Elution Buffer and incubate at 55°C for 4 min

Fig 1. Workflow of EpiArt Magnetic DNA Methylation Bisulfite Kit

8/Experiment Process

8-1/ Reagent Preparation

1. Add the specified volume of ethanol absolute to E-Wash Buffer before the first use (NB-54-0375-01: 80 ml per tube; NB-54-0375-08: 120 ml per tube). Tightly cap the bottles to prevent ethanol evaporation.
2. Prepare CT Conversion Mix: Add 900 µl of Nuclease-free ddH_2O, 300 µl of CT Conversion Diluent, and 50 µl of CT Conversion Buffer to one tube of CT Conversion Powder. Vortex at room temperature for 5 - 10 min until dissolved. Each tube of CT Conversion Mix provides enough reagent for 10 treatments.
   ▲ It is normal that small amounts of precipitation in the CT Conversion Mix. In this case, the vortexing time may be increased to 15 min. Prepare the ready-to-use CT Conversion Mix before use. Unused portions may be stored at room temperature (15 ~ 25°C) for 24 h, 0 ~ 4°C for 1 week, or -30 ~ -15°C for 1 month. These stored reagents should be fully reconstituted before use. It is recommended that the reagents be heated at 37°C for 10 min, equilibrated to room temperature and vortexed. Keep CT Conversion Mix protected from light.
   ▲ Tightly cap the CT Conversion Buffer and CT Conversion Mix after use.

8.2 /Bisulfite Conversion

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

Components	Volume
CT Conversion Mix	130 µl
Input DNA (100 pg - 2 µg)	X µl
Nuclease-free ddH_2O	To 150 µl

   ▲ If the treated DNA is intended for library preparation, the input DNA may be fragmented to the main peak around 600 bp before bisulfite conversion.

2. Mix well by vortexing or pipetting, and briefly centrifuge to collect the reaction mix to the bottom of the tube.
3. lace the PCR tube into a PCR instrument and run the following program:

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

   ▲ Set the heating volume to ≥100 µl.

8-3/Purification

1. 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 up and down 6 - 8 times or vortexing at a low speed for 30 sec. Incubate at room temperature for 5 min.
   ▲ E-Binding Beads stored at 0 ~ 4°C should be equilibrated to room temperature before each use. Since E-Binding Beads tend to settle, they should be mixed thoroughly by vortexing before each use.
2. Pulse spin the 1.5 ml Nuclease-free centrifuge tube. Place the tube on a magnetic rack until the solution becomes clear (about 3 min), then carefully remove and discard the supernatant.

3. Add 400 µl of E-Wash Buffer (pre-mixed with ethanol absolute) to the 1.5 ml Nuclease-free centrifuge tube. Mix well by pipetting up and down 6 - 8 times or vortexing at a low speed for 30 sec. Pulse spin the tube and place it on the magnetic rack until the solution becomes clear (about 3 min), and then carefully remove and discard the supernatant.
4. Add 200 µl of E-Desulphonation Buffer to the 1.5 ml Nuclease-free centrifuge tube. Mix well by pipetting up and down 6 - 8 times or vortexing at a low speed for 30 sec. Incubate at room temperature (15 ~ 25°C) for 15 min. Pulse spin the tube and place it on the magnetic rack until the solution becomes clear (about 3 min), and then carefully remove and discard the supernatant.
   ▲ Do not keep the sample in the E-Desulphonation Buffer for more than 25 min. This includes the time for bead resuspension, incubation, and supernatant removal.
5. Add 400 µl of E-Wash Buffer (pre-mixed with ethanol absolute) to the 1.5 ml Nuclease- free centrifuge tube. Mix well by pipetting up and down 6 - 8 times or vortexing at a low speed for 30 sec. Pulse spin the tube and place it on the magnetic rack until the solution becomes clear (about 3 min), and then carefully remove and discard the supernatant.
6. Repeat Step 5 once and discard the supernatant for a total of two washes.
   ▲Use a 10 µl pipette to remove all the residual liquid to shorten the drying time.
7. Dry at 55°C for 5 - 15 min or at room temperature for 20 - 30 min until all the residual liquid is removed and the beads appear matte.
   ▲ Air dry the beads thoroughly as the residual liquid may interfere with downstream experiments. The beads will change in appearance from glossy black when still wet to a dull brown when fully dry.
8. Add 25 µl of E-Elution Buffer. Resuspend the beads by pipetting up and down 6 - 8 times or vortexing at a low speed for 30 sec. Incubate at 55°C for 4 min. Pulse spin the tube and place it on the magnetic rack until the solution becomes clear (about 3 min). Transfer the supernatant to a clean 1.5 ml Nuclease-free centrifuge tube.
9. Store the bisulfite-converted DNA at -30 ~ -15°C. For long term storage, store at -85 ~ -65°C. Avoid repeated freeze-thaw cycles.

9/FAQ & Troubleshooting

Question	Solutions
What are the requirements for input DNA?	Nuclease-free ddH_2O or TE can be used to dissolve the DNA. The input amount can range from 100 pg to 2 µg, and the volume should be 20 µl. The A260/A280 ratio should be between 1.6 and 2.0.
Can I increase the volume of beads to improve DNA recovery?	Increase the volume of beads as appropriate can improve the DNA recovery rate. It is recommended using no more than 15 µl of beads. This operation may cause the beads to be pipetted when transferring the supernatant after elution, so be careful. If the beads are pipetted when collecting the supernatant, place the eluate containing the beads on the magnetic rack, and pipette the supernatant again.
How can I streamline the process when working with a large number of samples?	When purifying the bisulfite-converted DNA (**8-3/Purification/Step 3**), you can resuspend the beads in 200 µl of E-Wash Buffer and transfer the solution to 8-strip tubes (transfer as much liquid as possible to avoid loss of product). The volume of E-Wash Buffer used after desulphonation may also be adjusted to 200 µl. The subsequent steps can be carried out using a multichannel pipette.

Question	Reasons	Solutions
Low DNA recovery	Input DNA contains impurities	Ensure a DNA A260/A280 ratio of 1.6 - 2.0.
	Not enough ethanol in E-Wash Buffer	Add the specified volume of ethanol absolute and do not leave uncapped for long periods of time.
	Not enough organic solvents in E-Desulphonation Buffer	Do not leave uncapped for long periods of time.
	Desulphonation time too long (over 25 min)	Limit desulphonation to 25 min.
Low conversion rate	Degraded CT Conversion Mix	Resuspend and store the CT Conversion Mix correctly and use within the shelf life.
	Wrong reaction temperature or time	Set the correct reaction temperature and time according to the Instructions for Use.
	GC-rich input DNA or suboptimal input amount	Extend bisulfite conversion time from 90 min (64°C) to 2.5 h (64°C).