EpiArt DNA Methylation Bisulfite Kit

Catalog# EM101

Version 9.2

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01/ Introduction

DNA methylation is closely related to gene expression and gene function, and plays a pivotal role in physiological and pathological processes such as genetic imprinting, embryonic development, chromosomal gene silencing and cell cycle regulation. Efficient and accurate detection of DNA methylation has become increasingly important for biology, genetics, pathology, pharmacology, and medical diagnosis. Bisulfite conversion is a common technique used to study DNA methylation that involves converting unmethylated cytosine bases to uracil bases by treating the DNA with bisulfite, while leaving 5-methylcytosine (5-mC) intact. After conversion, DNA methylation can be determined by PCR amplification or DNA sequencing.

This kit combines DNA denaturation (by thermal denaturation instead of chemical denaturation) and bisulfite conversion into one step, and the conversion reaction time is only 140 min; Using the on-membrane desulphonation technique to expand the amount of DNA input to 100 pg - 2 μg. The recovery efficiency is ≥ 80% and the unmethylated cytosine conversion efficiency is ≥ 99%. The converted product is suitable for downstream applications such as PCR amplification and NGS sequencing.

02/ Components

Components	EM101-01 (50 rxn)	EM101-02 (200 rxn)
CT Conversion Powder	5 × 10 rxn	20 × 10 rxn
CT Conversion Diluent	1 ml	4 × 1 ml
CT Conversion Buffer	500 μl	2 × 1 ml
E-Binding Buffer	30 ml	120 ml
E-Wash Buffer	20 ml	2 × 40 ml
E-Desulphonation Buffer	25 ml	100 ml
E-Elution Buffer	1 ml	4 × 1 ml
EpiArt DNA Columns	50 Tubes	4 × 50 Tubes
Collection Tubes	50 Tubes	4 × 50 Tubes

CT Conversion Powder: for conversion reaction.

CT Conversion Diluent: for diluting CT Conversion Powder.

CT Conversion Buffer: the dissolving solution of CT Conversion Powder.

▲Prepare CT Conversion Mix: add 1 ml of ddH2O, 200 μl of CT Conversion Diluent and 100 μl of CT Conversion Buffer to a tube of CT Conversion Powder. Dissolve completely and mix well.

E-Binding Buffer: providing binding condition of the DNA to the membrane.

E-Wash Buffer: removes residual salt ions in DNA

E-Desulphonation Buffer: removes sulfonic acid group.

E-Elution Buffer: elutes the binding converted DNA on the column.

EpiArt DNA Columns: specifically adsorbing converted DNA.

Collection Tubes: filtrate collection tube.

03/ Storage

Store at 15°C ~ 25°C. Transport at room temperature.

The dissolved CT Conversion Mix can be stored at room temperature (15°C ~ 25°C) for 24 h, frozen (-30°C ~ -15 °C) for 1 month;

Protect it away from light.

04/ Applications

This kit is suitable for DNA that has not been converted by bisulfite.

Input DNA range: 100 pg - 2 μg. 100 ng - 1 μg is recommended. Compatible with DNA templates from different sources:

1.DNA extracted from cells or tissues of animals, plants, microorganisms.

2.Cell-free DNA, cfDNA.

3.Purified PCR products.

05/ Additional Materials Required

1.5 ml sterile centrifuge tube, Absolute ethanol, Ultrapure sterile water (ddH2O).

06/ Notes

1.Prepare CT Conversion Mix:

Add 1 ml of ddH2O, 200 μl of CT Conversion Diluent and 100 μl of CT Conversion Buffer to a tube of CT Conversion Powder. Vortex at room temperature for about 1 min until dissolved. Each tube of CT Conversion Mix can be subjected to about 10 reactions.

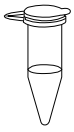
▲Appearing a small amount of precipitate is normal and does not affect the experimental results. It is recommended to use CT Conversion Mix freshly prepared. And the dissolved CT Conversion Mix can be stored at room temperature (15°C ~ 25°C) for 24 h, or at -30°C ~ -15°C for 1 month. It is necessary to equilibrate to room temperature and vortex to mix thoroughly before use.

2. Please add proper volume of absolute ethanol to E-Wash Buffer before use (Add 80 ml of absolute ethanol to E-Wash Buffer in EM101-01; add 160 ml of absolute ethanol to each bottle of E-Wash Buffer in EM101-02). After adding ethanol, tighten the lid tightly to prevent evaporation of ethanol.

3. E-Desulphonation Buffer contains volatile organic solvents, please tighten its lid tightly to prevent evaporation.

4. The products of conversion reaction can be stored at -20°C and stored at -70°C for long-term.

07/ Principle and Procedure

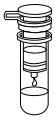


◇Add 130 μl CT Conversion Mix to 20 μl DNA (100 pg - 2 μg) and mix thoroughly.

◇Reaction procedure: 98°C 10 min; 64°C 40 min; 98°C 5 min; 64°C 40 min; 98°C 5 min; 64°C 40 min; 4°C hold.

◇Add 600 μl E-Binding Buffer to the EpiArt DNA Column.

◇Add 150 μl of the reaction solution from the previous step to the adsorption column which has been balanced by the E-Binding Buffer; cover the tube lid and mix by inverting for 8-10 times. Centrifuge at 12,000 × g for 30 - 60 sec, then discard the filtrate.



◇Add 500 μl of E-Wash Buffer (with absolute ethanol added) to the adsorption column. Centrifuge at 12,000 × g for 30 - 60 sec, then discard the filtrate.

◇Add 500 μl E-Desulphonation Buffer to the adsorption column, incubate at room temperature for 15 min, centrifuge at 12,000 × g for 30 - 60 sec, then discard the filtrate.

◇Add 500 μl of E-Wash Buffer (with absolute ethanol added) to the adsorption column. Centrifuge at 12,000 × g for 30 - 60 sec, then discard the filtrate.

◇Repeat cleaning once (last step).

◇Centrifuge empty adsorption column at 12,000 × g for 2 min. Transfer the adsorption column to a new collection tube, then place it at room temperature for 2 min to dry the membrane.

◇Add 10 μl - 20 μl of E-Elution Buffer. Incubate at room temperature for 1-2 min, centrifuge at 12,000 × g for 2 min, and collect the filtrate.

08/ Protocol

Please read this protocol carefully before starting the experiment.

08-1/ Reagent Preparation

1.Please add proper volume of absolute ethanol to E-Wash Buffer before first use (Add 80 ml of absolute ethanol to E-Wash Buffer in EM101-01; add 160 ml of absolute ethanol to each bottle of E-Wash Buffer in EM101-02). After adding ethanol, tighten the lid tightly to prevent evaporation of ethanol.

2.Prepare CT Conversion Mix:

Add 1 ml of ddH2O, 200 µl of CT Conversion Diluent and 100 µl of CT Conversion Buffer to a tube of CT Conversion Powder. Vortex at room temperature for about 1 min until dissolved. Each tube of CT Conversion Mix can be subjected to about 10 reactions.

▲It is recommended to use CT Conversion Mix freshly prepared.

And the dissolved CT Conversion Mix can be stored at room temperature (15°C ~ 25°C) for 24 h, or at -30°C ~ -15°C for 1 month. It is necessary to equilibrate to room temperature and vortex to mix thoroughly before use.

08-2/ Bisulfite Conversion

1.Equilibrate CT Conversion Mix to room temperature and prepare the reaction solution in a 200 µl sterile PCR tube as follows:

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

2.Mix the tube by inverting or pipetting, then centrifuge briefly to collect the reaction solution to the bottom of the tube.

3.Put the tube in a PCR instrument and run the following PCR program:

Temperature	Time
Hot lid of 105˚C	On
98˚C	10 min
64˚C	40 min
98˚C	5 min
64˚C	40 min
98˚C	5 min
64˚C	40 min
4˚C	Hold (< 24 h)

08-3/ Purification of Conversion Products

1.Assemble the EpiArt DNA Columns with the Collection Tubes.

2.Add 600 µl E-Binding Buffer to the EpiArt DNA Column, then add the reaction solution from Step 08-2/ to the adsorption column; gently mix the tube by inverting for 8-10 times to mix thoroughly the reaction solution with E-Binding Buffer.

3.Centrifuge at 12,000 × g for 30 - 60 sec, discard the filtrate and reuse the Collection Tube.

4.Add 500 µl of E-Wash Buffer (make sure that the absolute ethanol has been added before use) to the adsorption column. Centrifuge at 12,000 × g for 30 - 60 sec, discard the filtrate and reuse the Collection Tube.

5.Add 500 µl E-Desulphonation Buffer to the adsorption column, incubate at room temperature (15°C - 25°C) for 15 min, centrifuge at 12,000 × g for 30 - 60 sec, then discard the filtrate and reuse the Collection Tube.

6.Add 500 µl of E-Wash Buffer (make sure that the absolute ethanol has been added before use) to the adsorption column. Centrifuge at 12,000 × g for 60 sec, discard the filtrate and reuse the Collection Tube.

7.Repeat Step 6.

8.Centrifuge empty adsorption column at 12,000 × g for 2 min.

9.Transfer the adsorption column to a new 1.5 ml centrifuge tube, open the lid for 2 minutes and dry thoroughly. Add 10 µl -20 µl of E-Elution Buffer to the center of the adsorption column, then place it at room temperature for 1 - 2 min, centrifuge at 12,000× g for 2 min to collect DNA filtrate.

10.Discard the adsorption column. Store the DNA at -20°C and stored at -70 °C for long-term.

09/ Troubleshooting

FAQ	Solutions
Input DNA requirements	1. It is applicable for DNA dissolved in ddH2O, TE or other buffer. 2. The optimal amount of input DNA is 100 ng - 1 μg. Too low input is not conducive to downstream detection. Too high input may affect conversion efficiency. 3. The ratio of A260 / A280 should be between 1.7 and 1.9.
Reaction instrument requirements	The volume for heating should be ≥ 100 μl.

Problem	Possible Reason	Recommended Solutions
Low DNA Yield	1. Poor quality of initial DNA	Ensure that the A260 / 280 ratio of DNA is between 1.7 and 1.9; The DNA bands in gel electrophoresis are clear without degradation.
	2. Insufficient ethanol in E-Wash Buffer	Add the proper volume of absolute ethanol, DO NOT open the lid for a long time.
	3. Insufficient organic solvent in E-Desulphonation Buffer	DO NOT open the lid for a long time.
Low Conversion Efficiency	1. CT Conversion Mix is invalid.	Please prepare the CT Conversion Mix correctly according to the instructions and use it during its shelf life.
	2. Set the wrong temperature or reaction time.	Set the correct temperature and reaction time according to the instructions.
	3. Add excess input DNA, or DNA sample with high GC-content. DNA samples with high GC-content or excess DNA input.	Control the Input DNA within optimal range or extend the reaction time of 64°C to 50 min - 60 min.