Apostle MiniMax DNA Methylation Bisulfite Conversion Module User Manual

Thoroughly Read This Manual Before Operation.
For Research Use Only.

Statement

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

This instruction is intended for use with the MiniMax DNA Methylation Bisulfite Conversion Module.

Content

Announcement

Environment

- Temperature: 20-25°C; Humidity: 40-60%.
- The personal protective equipment should be equipped during the experiment period.
- Bisulfite Reagent can cause skin corrosion and eye irritation, and shall be used with caution and follow safety precautions.
- MiniMax Desulf Bufffer contains strong alkaline solution, and shall be used with caution and follow safety precautions.

Temperature Control

- This product contains components with different storage temperature requirements. All components should be stored strictly according to the instructions on the label.
- When the laboratory environment temperature is stable at 20-25°C, the preparation and operation of the reaction system during the conversion process can be conducted at room temperature.
- Make sure the thermal cycler reaches the designated temperature before loading any reaction tube in.

Avoid Cross-contamination

- Briefly centrifuge all the tubes to collect the contents to the bottom before opening the lids.
- Physically separate the laboratory space, equipment and consumables among different steps.
- After the conversion is complete, promptly change to new gloves and replace the working surface.
- Clean the lab area using 0.5% sodium hypochlorite once finishing the experiment.

Bisulfite Conversion

- Bisulfite Reagent should be freshly prepared and stored in a light-protected manner. The maximum number of times the container can be opened during use should not exceed 3 times. After each use, the container should be tightly wrapped with a sealing film. The freshly prepared Bisulfite Reagent has a shelf life of 15 days.
- Bisulfite Reagent may exhibit slight precipitation, which is a normal phenomenon. It should be mixed thoroughly before use, and it does not affect the experimental results.
- 30% Desulf Buffer should be freshly prepared.
- MiniMax Bisulfite Beads are quite viscous and need to be thoroughly vortexed before use.

Others

- Unless otherwise specified, all the centrifuge steps are conducted at room temperature (20-25°C).
- Unless otherwise specified, all mixing steps listed as "mix thoroughly" should be performed by either vortexing for 10 sec or pipetting up and down for 10 times, and then briefly centrifuge to collect the contents.

Introduction

Apostle MiniMax DNA Methylation Bisulfite Conversion Module is a methylation conversion reagent designed for DNA samples. The product has been designed based on the principle of bisulfite conversion, where non-methylated cytosine are converted to uracil while methylated cytosine remain unchanged. The kit utilizes magnetic bead purification, offering a simple operation and a short processing time. It is compatible with automation workstations and can be combined with the MiniMax Methylation Library Preparation Module to achieve rapid construction of methylated DNA libraries.

All components of this kit have undergone quality control and functional verification. The conversion efficiency of non-methylated cytosine is >99%, supporting various types of DNA samples, including gDNA, cfDNA, and FFPE-derived DNA (Grade B). The recommended input range for this kit is 10-1000 ng of high-quality DNA. When used in conjunction with the MiniMax Methylation Library Preparation Module (double-stranded or single-stranded), the resulting methylated libraries can be directly sequenced or used for downstream targeted methylation library construction.

Kit Content

This instruction is intended for use with the Apostle MiniMax DNA Methylation Bisulfite Conversion Module with the following components:

Catalog#	Item	Package/Storage
1002701	MiniMax DNA Methyl Bisulfite Conversion Module, 24 rxn	Box 1/10~30°C Box 2/2~8°C
1002702	MiniMax DNA Methyl Bisulfite Conversion Module, 96 rxn	Box 1/10~30°C Box 2/2~8°C

Package#	Component	Volume 1002701 24 rxn	Volume 1002702 96 rxn	Storage
Box 1	MiniMax Bisulfite Diluent	4.5 mL	9 mL	10~30°C
	MiniMax Bisulfite Mix	4×1 Tube	8×1 Tube	10~30°C
	MiniMax Desulf Buffer	1.8 mL	7.5 mL	10~30°C
Box 2	Nuclease Free Water	2.5 mL	8 mL	2~8°C
	MiniMax Bisulfite Beads	4.8 mL	3×6.2 mL	2~8°C
	MiniMax DNA Protect Buffer	100 µL	380 µL	2~8°C

Recommended Product

Item	Description	Detail	Catalog#
Methyl Lib Prep Module	MiniMax Methyl Library Preparation Module, 24 rxn	24 rxn	1002501
	MiniMax Methyl Library Preparation Module, 96 rxn	96 rxn	1002502
Methyl Adapter (SI) Module	MiniMax Methyl Adapter (SI) Module Set A1 (for MGI), 24 rxn	#1-12	1003631
	MiniMax Methyl Adapter (SI) Module Set B1 (for MGI), 96 rxn	#1-24	1003632
Methyl Adapter (MDI) Module	MiniMax Methyl Adapter (MDI) Module Set A1 (for MGI), 24 rxn	#1-12	1003740
	MiniMax Methyl Adapter (MDI) Module Set B1 (for MGI), 96 rxn	#1-24	1003741
	MiniMax Methyl Adapter (MDI) Module Set B2 (for MGI), 96 rxn	#25-48	1003742
Methyl Stubby Adapter (UDI) Module	MiniMax Methyl Stubby Adapter (UDI) Module Set A1 (with 10 nt Index), 24 rxn	#1-12	1003371
	MiniMax Methyl Stubby Adapter (UDI) Module Set B1 (with 10 nt Index), 96 rxn	#1-24	1003381
	MiniMax Methyl Stubby Adapter (UDI) Module Set B2 (with 10 nt Index), 96 rxn	#25-48	1003382

Equipment and Consumable

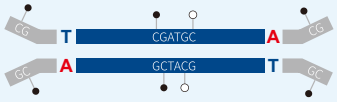
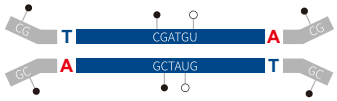
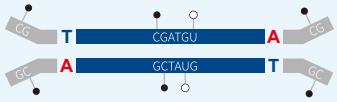
Equipment

Item	Description
Pipettor	General laboratory supplier
Thermal cycler	General laboratory supplier
Benchtop centrifuge	General laboratory supplier
Microcentrifuge	General laboratory supplier
Vortex mixer	General laboratory supplier
Magnet stand	Thermo Fisher DynaMagTM-2 Magnet (Cat # 12321D) Thermo Fisher DynaMagTM-96 Side Magnet (Cat#12331D) BORTHEE-96 Side Magnet (Cat # MAG-96-11) or equivalent
Heated constant temperature metal bath	General laboratory supplier
Timer	General laboratory supplier

Consumable

Item	Description
Absolute Ethanol	General laboratory supplier, analytical grade
0.2 mL PCR Tube	Axygen MAXYMum Recovery™ PCR Tubes, 0.2 mL flat cap (Cat # PCR-02-L-C) or equivalent

Workflow

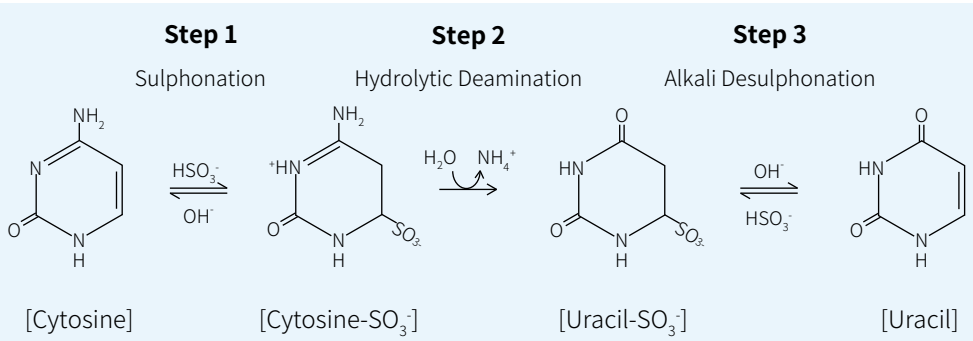
Product of Adapter Ligation	Fragmented gDNA/cfDNA	Workflow	Duration
			
		Bisulfite Conversion	70-115 min
		Desulfonation and Purification Recovery	30 min

Methylated C
Unmethylated C

Methylated C
Unmethylated C

The above schematic diagram of adapter-ligated product is taking MiniMax Methyl Stubby Adapter (UDI) as an example.

BS Conversion Principle



Protocol

Sample Type

1. For the double-stranded methylation library preparation approach, this kit is used in conjunction with the MiniMax Methylation Library Preparation Module. It is designed to convert the adapter-ligated products, followed by PCR amplification of the converted products. The recommended input range for library preparation is 10-1,000 ng.

2. For the single-stranded methylation library preparation approach, this kit is used in conjunction with the MiniMax Single-Stranded Methylation Library Preparation Module. It is designed to convert the original DNA samples into single-stranded converted products. The recommended input range for conversion is 10-500 ng.

Reagent Preparation

Item	Description	Note
Bisulfite Reagent*	Add 1 mL of MiniMax Bisulfite Diluent to the Bisulfite Mix after brief centrifugation for 1-2 sec.	Incubate the Bisulfite Reagent at 60°C for 15 min, and vortex every 5 min.
30% MiniMax Desulf Buffer*	Prepare 30% MiniMax Desulf Buffer according to the required volume for each reaction: n × 60 µL MiniMax Desulf Buffer + n × 140 µL ethanol (n is the number of reactions).	Always prepare fresh
MiniMax Bisulfite Beads	Equilibrate at room temperature for 30 min	
MiniMax DNA Protect Buffer	Equilibrate at room temperature for 5 min	

Note: *The reagents are freshly prepared. Bisulfite Reagent should be stored at room temperature, protected from light. The total number of times the cap is opened during use should not exceed 3 times. After each use, tightly wrap the tube opening with a sealing film. The freshly prepared Bisulfite Reagent has a shelf life of 15 days. Some precipitation in the Bisulfite Reagent is normal and does not affect product performance. Prior to each use, it must be incubated at 60°C for at least 5 min, vortexed to mix thoroughly, and then centrifuged briefly before use.

Step 1: Bisulfite Conversion

1. Set up each reaction in a 0.2 mL PCR tube as follows:

Product of Adapter Ligation/Fragmented DNA ❗	11 µL
Bisulfite Reagent	27 µL
MiniMax DNA Protect Buffer	2 µL
Total	**40 µL**

❗ **Note: When using the double-stranded methylation library preparation protocol, it corresponds to methylated adapter-ligated products. When using the single-stranded methylation library preparation protocol, it corresponds to fragmented DNA samples.**

2. Mix thoroughly and briefly centrifuge to collect all reaction solution to the bottom of the PCR tube.
3. Perform the following procedures on the thermal cycler and make sure the thermal cycler is stabilized at 90°C before loading the tubes:

Temperature	Time	Cycles
95°C	5 min	-
75°C	20 min	-
95°C 75°C	5 min 40 min	1 Cycle for normal samples; 2 cycles for FFPE samples.
20°C	Hold	-

❗ **Note: The heated lid shall be set at 105°C.**

STOP **Safe Stopping Point:** The product after bisulfite conversion can be stored overnight at 4°C, but avoid long-term storage.

Step 2: Desulfonation and Purification Recovery

Reminder: 70% ethanol should be freshly prepared.

1. Add 160 µL of MiniMax Bisulfite Beads (equilibrated for 30 min at room temperature) to the reaction system from Step 1, mix thor-oughly by pipetting or vortexing, briefly centrifuge for 1-2 sec, and then incubate at room temperature for 5 min (do not ce-ntrifuge the magnetic beads to the bottom of the tube).

Note: MiniMax Bisulfite Beads are viscous and need to be v mixed thoroughly prior to use. When pipetting, take and release the solution slowly and steadily to ensure accurate volume.

2. Place the PCR tube on the magnet stand for 15 min until the solution is completely clear. Carefully remove and discard the supernatant without disturbing the beads.

3. Remove the PCR tube off the magnet stand, add 200 µL of freshly prepared 70% ethanol. Mix by pipetting or vortexing and then place the tube on the magnet stand after brief centrifugation until the solution is complete clear. Carefully remove and discard the supernatant without disturbing the beads.

Reminder: If the beads are difficult to resuspend, remove the PCR tube from the magnetic stand and allow to stand at room temperature for 1 min before resuspending the beads (same for the subsequent steps).

4. Remove the PCR tube off the magnet stand, add 180 µL of freshly prepared 30% MiniMax Desulf Buffer to each reaction solution Mix by pipetting or vortexing and then place the tube on the magnet stand after brief centrifugation, stand at room temperature for 5 min until the solution is completely clear. Carefully remove and discard the supernatant without disturbing the beads.

Note: 30% MiniMax Desulf Buffer should be freshly prepared. Please refer to the reagent preparation instructions for the preparation protocol.

5. Remove the PCR tube off the magnet stand, add 180 µL of freshly prepared 70% ethanol to each reaction solution. Mix by pipetting or vortexing and then place the tube on the magnet stand until the solution is completely clear. Carefully remove and discard the supernatant without disturbing the beads.

6. Repeat step 5 once.

7. Briefly centrifuge and then place the PCR tube on the magnet stand. Carefully remove all residual ethanol without disturbing the beads by using a 10-µL pipette tip.

8. Dry the beads on the magnet stand at room temperature for 2 min.

9. Remove the PCR tube off the magnet stand. Add 21 µL of Nuclease Free Water to the PCR tube, mix gently by pipetting or vortexing and keep it stand at room temperature for 2 min.

10. Briefly centrifuge and place the tube on the magnet stand for 2 min until the solution is completely clear. Transfer the clear supernatant to a new 0.2 mL PCR tube. Avoid transfer of Beads.

Note: For the double-stranded library preparation protocol, the converted product can be used for PCR amplification or stored at -20°C with beads for up to 1 week. For the single-stranded library preparation protocol, the converted product can be used for single-stranded library preparation or stored at -20°C for up to 1 week.

Appendix： Frequently Asked Question and Solution

Question	Possible reason	Solution
Incomplete conversion	The library after conversion is contaminated with unconverted library.	Use filter-tipped pipette tips; After conversion, promptly replace with new latex gloves, a new work surface, and new pipettes.
	Bisulfite Reagent is prone to oxidation and crystallization, leading to a decrease in sulfite solution concentration, which can affect the conversion efficiency.	Use Bisulfite Reagent within its expiration date and limit the exposure time of Bisulfite Reagent to air.
Low recovery rate of conversion product	Inaccurate input amount.	The actual input amount is lower than expected. It is recommended to use Qubit for precise quantification.
	Discard a portion of the beads during the purification process.	When using vortex mixing, ensure that the tube cap is tightly closed. Do not discard the beads during the supernatant removal process.

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