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DNA Methylation Library Prep Kit for Illumina

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Pack Size: 24 rxns

Ver1.0

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Description

DNA Methylation Library Prep Kit for Illumina is designed for the Illumina high-throughput sequencing platform. It converts methylated DNA into NGS sequencing libraries using a single-strand ligation technique. Compatible with DNA samples as short as 40bp. It is easy to use, suitable for automated library preparation, and can be completed in 2 hours. This kit undergoes strict quality control to ensure the quality and efficiency of library preparation.

Material provided:

1. Dilution Buffer: 5 ml
2. 3' Ligation Buffer: 240 ul
3. 3' Ligation Enzyme Mix: 120 ul
4. 3' Adapter: 120 ul
5. Extension Primer: 120 ul
6. Extension Enzyme Mix: 840 ul
7. 5' Ligation Mix: 360 ul
8. 5' Adapter: 120 ul
9. 2X HIFI PCR Mix V3: 600 ul

Notes:

1. If the DNA sample is fragmented, such as ChIP DNA or cfDNA, no fragmentation step is needed. If the DNA sample is intact genomic DNA, fragmentation is recommended.
2. The protocol does not include size selection. If needed, you may choose between library size distribution (with dual round bead size selection) and library complexity (without size selection).
3. The 3' Adapter and 5' Adapter are partial adapters. The Index and P5/P7 sequences must be introduced into the library molecules during the subsequent amplification step.
4. All components should be thawed at room temperature, mixed thoroughly, and placed on ice before use.
5. When preparing the reaction mixtures, use a pipette to mix gently to avoid vortexing, which may decrease the library yield.

Experimental Workflow:

1. DNA Denaturation:

1. Preheat the PCR instrument: Set the heat lid to 105°C and the reaction temperature to 95°C. Prepare the 3' Adapter Ligation premix (step 2) and keep on ice.
2. In a new nuclease-free PCR tube, prepare the following mixture:

Reagents	Volume
Input DNA	X ul
Dilution Buffer	To 20 ul

3. Heat the mixture at 95°C for 2 min and then quickly cool it on ice for 2 min. Proceed to the next step immediately.

2. Adapter Ligation:

1. Thaw the 3' Ligation Buffer, 3' Ligation Enzyme Mix, and 3' Adapter at room temperature, invert to mix, and keep on ice.

Reagents	Volume
3' Ligation Buffer	10 ul
3' Ligation Enzyme Mix	5 ul
3' Adapter	5 ul
Total	20 ul

2. Add 20 ul of the premix to 20 ul of denatured DNA and mix gently with a pipette. Briefly centrifuge and

collect the mixture at the bottom of the tube.

3. Place the PCR tube in the PCR instrument to perform the following reaction:

Temperature	Time
Heat lid 105°C	ON
37°C	15 min
95°C	2 min
4°C	∞

3. Extension:

1. Thaw the Extension Primer and Extension Enzyme Mix at room temperature and keep on ice.
2. In a nuclease-free PCR tube, prepare the following mixture:

Reagents	Volume
Previous Reaction Mixture	40 ul
Extension Primer	5 ul
Extension Enzyme Mix	35 ul
Total	80 ul

3. Mix gently and briefly centrifuge. Place the PCR tube in the PCR instrument and run the following reaction:

Temperature	Time
Heat lid 105°C	ON
98°C	1 min
62°C	2 min
65°C	5 min
4°C	∞

4. 5' Adapter Ligation:

1. Thaw the 5' Ligation Mix and 5' Adapter at room temperature and mix thoroughly.
2. Prepare the following mixture:

Reagents	Volume
Previous Reaction Mixture	20 ul
5’ Ligation Mix	15 ul
5’ Adapter	5 ul
Total	40 ul

3. Gently mix with a pipette and briefly centrifuge to collect the reaction mixture at the bottom of the tube.
4. Place the PCR tube in the PCR instrument and run the following reaction:

Temperature	Time
Heat lid 105°C	ON
25°C	15 min
4°C	∞

5. Library Amplification:

Amplify the library using primers to generate a complete library.

1. Prepare the following reaction in a sterilized PCR tube:

Reagent	Volume
Previous purified product	20 ul
2X HIFI PCR Mix V3	25 ul
Index Primers	5 ul
Total	50 ul

2. Amplify with the following conditions:

Temperature	Time	Cycles
Heat lid 105°C	ON	-
95°C	3 min	-
98°C	20 sec	2-23
60°C	5 sec	
72°C	5 min	-
4°C	∞	-

6. Library Quality Control:

1. Library Length Distribution Detection:
You can use electrophoresis-based devices such as LabChip GX, GXII, GX Touch (PerkinElmer), Bioanalyzer, Tapestation (Agilent Technologies), or Fragment Analyzer (Advanced Analytical) to detect the library length distribution.

2. Library Concentration Detection:
We recommend using a qPCR-based method for absolute quantification. Additionally, the library concentration can be determined using fluorescence dyes that specifically bind to double-stranded DNA, such as the Qubit method.

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