ELLIS BIO

User Manual
SuperMethyl™ Max Bisulfite Conversion Kit
24 Reactions, Magnetic Bead Purification

MAX-24R-BEAD

Version 2026.6.1

Questions? We're ready to help!

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1. Key Features

Ellis Bio is committed to revolutionizing DNA methylation analysis with unparalleled speed and precision.

- ✓ **Ultra-Mild Bisulfite Conversion:** Achieve complete and ultra-mild bisulfite conversion to minimize DNA damage.
- ✓ **High Efficiency:** Consistently delivers 99.8% C-to-T conversion rates of unmethylated cytosines for accurate analysis while preserving methylated cytosines.
- ✓ **High Compatibility with low-input DNA:** Minimized DNA damage enables robust application to ultra-low input, with inputs ranging from 100 pg to 2 µg of gDNA or cfDNA.
- ✓ **Low Background Noise:** Reduces false positives and enhances the accuracy of 5mC signal detection.
- ✓ **Versatile Applicability:** Ideal for a wide range of applications, including methylation-specific PCR (MSP), microarrays, and NGS.

This kit offers an unmatched combination of maximized DNA integrity, accuracy, and reliability, setting a new benchmark in DNA methylation analysis.

2. Product Description

The **SuperMethyl™ Max Bisulfite Conversion Kit** from Ellis Bio offers the exceptionally mild yet powerful bisulfite conversion for DNA methylation analysis. Featuring a newly engineered **ultra-mild bisulfite conversion formulation** and **a magnetic bead-based DNA purification method**, this kit simplifies the bisulfite conversion workflow and achieves efficient conversion with minimal DNA degradation. Complete bisulfite conversion and DNA purification can be accomplished in 3 hours or less, preserving DNA integrity even with ultra-low input samples, while consistently achieving **99.8%** conversion efficiency. Its broad applicability makes it a valuable tool for both research and translational settings.

Designed for compatibility and consistent performance in sensitive and/or low-input DNA samples, the SuperMethyl™ Max Bisulfite Conversion Kit delivers reliable, reproducible results, making it the preferred choice for precision-focused researchers.

Minimal DNA damage: Ultra-Mild Bisulfite Technology preserves DNA integrity, ideal for cfDNA and low-input samples.

Exceptional C-to-T Conversion Efficiency: Users typically achieve 99.8% conversion rates.

High Library Complexity: Produces libraries with much greater complexity and larger insert sizes compared to conventional bisulfite conversion methods.

Enhanced CpG Detection: Detects more CpG sites with fewer sequencing reads.

Uniform GC Coverage: Delivers consistent coverage across GC-rich and GC-poor regions.

Wide Input Range: Compatible with 100 pg to 2 µg of DNA input.

Low Background Noise: Reduces false positives and enhances the accuracy of 5mC signal detection.

Versatile DNA Fragmentation Compatibility: Supports both sonication and enzymatic fragmentation workflows.

3. Kit Components

Number of tests per kit: 24 reactions

Your Kit includes:

Component	Volume/Quantity	Storage
Kit Capacity	24 reactions	Please see storage recommendations for individual components
Conversion Reagent ◉	2 mL	4 °C (recommended)
Preparation Reagent ◉	120 µL	Room temperature
Enhancer A ◉	120 µL	Room temperature
Enhancer B ◉	240 µL	4 °C (recommended)
Binding Buffer	22 mL	Room temperature
Wash Buffer (concentrate)*	7 mL*	Room temperature
Desulphonation Buffer	6 mL	Room temperature
Elution Buffer	1 mL	Room temperature
Purification Beads ◉	400 µL	Room temperature
* Wash Buffer requires the addition of 28 mL 100% ethanol (EtOH) before first use.		

4. Notes for Users

User-supplied materials

- 100% ethanol
- Nuclease-free H_2O
- 1.5 mL low-adhesion microcentrifuge tubes and PCR tubes.
- Microtube rotator
- Magnetic tube racks
- Positive and negative control samples such as unmethylated DNA and fully methylated pUC19 DNA
- Lambda DNA (dam-, dcm-)

Storage

We recommend storing **Conversion Reagent** ◉ and **Enhancer B** ◉ at 4 °C and avoid exposure to air and light. All other kit components can be stored at room temperature. The kit is stable for up to 12 months. Please refer to the product label for the expiration date.

Applications

The kit is compatible with DNA from various sources including cell-free DNA (cfDNA), genomic DNA (gDNA) extracted from cells or tissues, and DNA derived from formalin-fixed paraffin-embedded (FFPE) samples. Other DNA sources might be compatible; we encourage users to run quality control tests on samples to confirm.

Input DNA Requirements

The kit requires an input DNA amount of 100 pg** to 2 µg. We advise quantifying the DNA with a precise instrument such as a Qubit Fluorimeter (Thermo).

**Users may be able to use inputs lower than 100 pg. We recommend users run quality control tests on samples that are outside the recommended range.

Product Performance Indicators

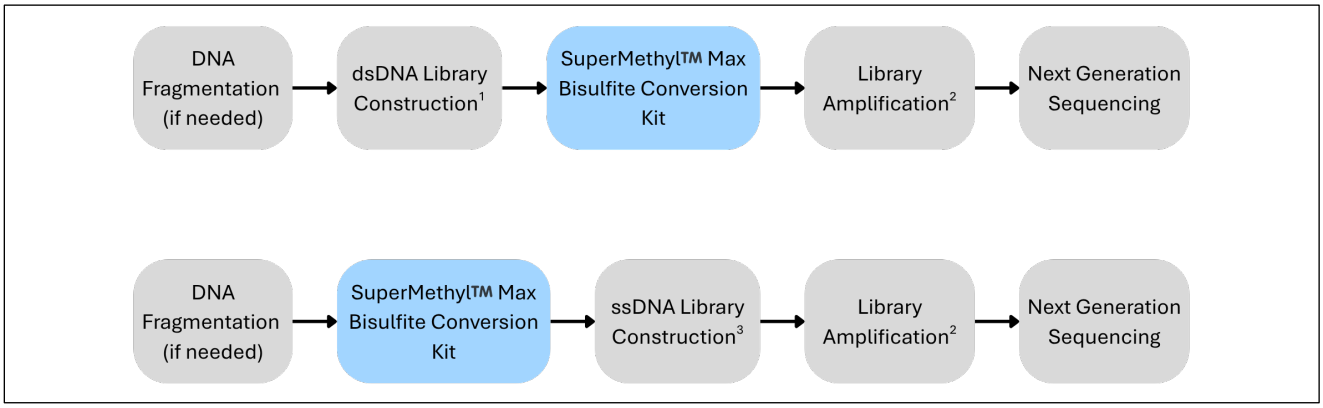
The C-to-T conversion rate at all unmethylated cytosines in both CpG and non-CpG contexts of λDNA exceeds 99.5%. The apparent methylation levels across CpG sites in fully methylated pUC19 control DNA are consistently greater than 95.0%, indicating effective preservation of methylated cytosines under the validated conditions.

Caution

This kit is for research use only and is not intended for use in diagnostic applications. **Preparation Reagent** ◉**, Conversion Reagent** ◉**, Enhancer A** ◉**, Enhancer B** ◉**, Desulphonation Buffer**, and **Wash Buffer** contain volatile ingredients. Cap the bottles tightly after use and store at recommended temperatures. Safety Data Sheets are available upon request.

5. Experimental Planning

1. If your application requires next-generation sequencing library preparation, the bisulfite conversion protocol can occur downstream or upstream of library preparation, depending on your experimental design.



[1] Available through Ellis Bio or other dsDNA library preparation suppliers. Use with methylated adapters.
[2] Use uracil-tolerant library amplification system, available through Ellis Bio or other suppliers.
[3] Available from other suppliers.

Kit Compatibility

We recommend testing quality control samples with your preferred library construction kit to determine compatibility. The SuperMethyl™ Max Bisulfite Conversion Kit is compatible with the following library preparation kits:

- Pre-bisulfite conversion:
  - Ellis Bio SuperMethyl™ dsDNA Library Kit for Illumina
  - KAPA EvoPrep Kit (Roche)
  - KAPA HyperPrep Kit (Roche)
  - NEBNext® Ultra™ II Ligation Module (New England Biolabs)

- Post-bisulfite conversion:
  - SRSLY® PicoPlus Uracil+ Kit (Claret Bioscience)
  - SRSLY® NanoPlus Uracil+ Kit (Claret Bioscience)
  - SRSLY® MethylPlus Kit (Claret Bioscience)
  - xGen™ Methylation-Sequencing DNA Library Preparation Kit (IDT)

Methylation controls

It is recommended to include appropriate positive and negative controls in the experiment. Spike-in controls such as unmethylated lambda DNA and methylated pUC19 DNA are useful options.

6. Experimental Protocol

1. Reagent Preparation

1.1. **Prepare Wash Buffer:** Add 28 mL of 100% ethanol (EtOH) to the **Wash Buffer** before first use. Invert to mix thoroughly and ensure the bottle cap is tightly sealed to prevent ethanol evaporation, which could impact the effectiveness of the **Wash Buffer**.

1.2. **Prior to using materials in 2 mL screwcap vials:** For any kit component provided in a 2 mL screwcap vial (*e.g.*, **Conversion Reagent ◉, Purification Beads ◉, Elution Buffer, etc.**), briefly centrifuge the vial to remove material that may have pooled in the cap. Use a micropipette to gently resuspend the contents so that all settled material is evenly mixed before use.

1.3. **Conversion Reagent ◉ Notes**: Immediately prior to use, please inspect the **Conversion Reagent ◉** for any signs of crystallization. If crystals are present, heat the vial at 55 °C or vortex until fully dissolved, then allow the reagent to equilibrate to room temperature before use. Failure to dissolve the crystals may significantly impair conversion efficiency. Minor crystallization on the vial cap is normal.

2. Sample Preparation

2.1. **For gDNA or FFPE-derived DNA:** Fragment the DNA using either sonication or enzymatic digestion. Purify the fragmented DNA using magnetic beads and perform size selection. Submit the processed DNA for quality control (QC) and determine the sample concentration.[1]

For cfDNA: Fragmentation is not required.[2]

⚠ *[1]Note: Refer to Section 5 for guidance on including positive and negative methylation controls. We also recommend using a fragment analysis method, such as the TapeStation (Agilent), to assess fragment size distribution. For accurate DNA quantification, use a fluorometric method such as the Qubit assay (Thermo Fisher Scientific).*

⚠ *[2]Note: Please see our Supplementary Section 7 about gDNA contamination in cfDNA for tips for clean-up.*

2.2. **Library preparation (see Section 5 above):** If your workflow involves a double-stranded DNA (dsDNA) library preparation, as illustrated in the figure in Section 5, proceed with the library construction steps: perform end repair, dA-tailing, ligation of methylated adapters, and carry out the post-ligation purification steps according to the manufacturer's protocol. **Do not begin library amplification at this stage**.

3. Bisulfite Conversion

3.1. In a 0.2 mL nuclease-free PCR tube, pipette the volume to obtain 100 pg – 2 µg of input DNA. Add nuclease-free H_2O up to a total volume of 20 µL[3].

⚠ *[3] Note: SuperMethyl Max kit is also compatible with larger DNA input volume (20-40 µL), please reach out to support@ellisbio.com for more information.*

3.2. Add 2 µL of **Preparation Reagent** ◉ to the tube per **Protocol Table 1**. Mix thoroughly by vortexing or gentle pipetting. Briefly centrifuge to collect the liquid at the bottom of the PCR tube.

Protocol Table 1	
Component	**Volume**
Input DNA	20 µL (100 pg - 2 µg)
Preparation Reagent ◉	2 µL
Total Volume	22 µL

3.3. Place the capped PCR tube in a thermal cycler and incubate at 42 °C (with a 55 °C heated lid) for 20 minutes using the program shown in **Protocol Table 2**. After the incubation, transfer the sample immediately to ice for 2 minutes, then allow it to equilibrate to room temperature.

Protocol Table 2		
Step	**Temperature**	**Time**
1. Sample prep incubation	42 °C	20 min
2. Transfer immediately to ice for at least 2 minutes		

3.4. In a 1.5 mL nuclease-free microcentrifuge tube, prepare the **Conversion Master Mix**[4] by adding components in the following order: first, add **Conversion Reagent** ◉, then add **Enhancer A** ◉ and vortex immediately for 10 seconds. Next, add **Enhancer B** ◉ and vortex for an additional 10 seconds. Refer to **Protocol Table 3** for component volumes:

Protocol Table 3	
Component (add in the order listed below)	**Volume**
Conversion Reagent[4] ◉	77 µL
Enhancer A ◉	2 µL
Enhancer B ◉	8 µL
Total volume	87 µL

⚠ *[4] Note: See Step 1.3. above for an important note regarding crystallization of the Conversion Reagent. We recommend preparing the Conversion Master Mix after Step 3.3.* ***If the Conversion Reagent has been stored at 4 °C, incubate the vial at 55 °C for 20 minutes before use. We recommend using the Conversion Master Mix immediately after its preparation.*** *However, if the Conversion Master Mix is not used immediately after its preparation, incubate it at 55 °C to minimize the risk of precipitation prior to Step 3.5.*

3.5. Prepare the bisulfite conversion reaction according to **Protocol Table 4**. After adding the **Conversion Master Mix**[5] to the **Prepared DNA** from Step 3.3 for each sample, **immediately mix thoroughly by pipetting 10 times**. Briefly centrifuge to collect liquid at the bottom of the PCR tubes. If the sample volume exceeds the thermocycler settings, please split the sample into additional PCR tubes as needed.

Protocol Table 4	
Component	**Volume**
Post-sample Preparation DNA (Step 3.3)	22 µL
Conversion Master Mix (Step 3.4)	87 µL
Total volume	109 µL

⚠ [5]*Note: Check the Conversion Master Mix for visible precipitation before use. If precipitation is observed, prepare a fresh Conversion Master Mix.* ***Following addition of the Conversion Master Mix to the post-sample preparation DNA, mix by pipetting up and down 10 times to ensure homogeneity immediately. For plate-based conversion incubations, vortex mixing is not permitted. Vortexing can cause the master mix to come into contact with the sealing film, potentially resulting in substantial precipitate formation.*** *The resulting precipitation may negatively impact conversion efficiency and compromise downstream cleanup and purification performance.*

3.6. Place the capped PCR tubes in a thermal cycler and incubate at 55 °C (with a 75 °C heated lid) using the following program in **Protocol Table 5.**

Protocol Table 5		
Step	**Temperature**	**Time**
1. Bisulfite conversion	55 °C	90 mins
2. Hold[6] (Optional)	20 °C	< 5 mins
3. Proceed to next step immediately[7]		

⚠ [6]*Note: This step is optional;* ***we recommend proceeding directly to the next step.*** *Please do not place the sample on ice or store it at 4 °C, as this may lead to white crystallization formation, which can reduce the DNA recovery rate.*

⚠ [7]*Note:* ***After conversion, proceed immediately to Step 4.2. Do not vortex the converted reaction before adding it to the Binding Buffer and Purification Beads, as this may cause the solution to become cloudy or form precipitates,*** *which can negatively impact the efficiency of the subsequent cleanup and purification process.*

4. Purification of Bisulfite-converted DNA

4.1. Add 800 µL of **Binding Buffer** and 15 µL of **Purification Beads**[8] to a 1.5 mL low-adhesion microcentrifuge tube.

⚠ [8]*Note: Purification Beads settle quickly, pipette or vortex thoroughly to keep them well-suspended. When working with multiple samples manually, we recommend vortexing the Purification Bead and Binding Buffer suspension frequently (for example, every 4 samples) to maintain consistent suspension.*

4.2. Transfer the bisulfite converted reaction mixtures from the PCR tubes (from Step 3.6) into the 1.5 mL microcentrifuge tube containing the **Binding Buffer** and **Purification Beads** ◉ (Step 4.1). Mix thoroughly by pipetting up and down or by gently vortexing at low speed for 10 seconds. Incubate at room temperature (15 - 25 °C) for 10 minutes with continuous rotation. **A microtube rotator or mixer is strongly recommended for this incubation step.**

4.3. Briefly centrifuge the microcentrifuge tube to collect the liquid and beads at the bottom, then place it on a magnetic stand for 3 minutes or until the supernatant becomes clear. While the microcentrifuge tube is on the magnetic stand, remove and discard the supernatant[9].

⚠ *[9]Note: If some Purification Beads adhere to the non-magnetic side of the tube, remove the supernatant slowly, allowing beads to migrate to the magnetic side as the liquid volume decreases.*

4.4. Remove the microcentrifuge tube from the magnetic stand for this step and each subsequent buffer addition.

4.5. Add 400 µL of **Wash Buffer** (ensure 100% ethanol was added before first use) to the beads and resuspend thoroughly by gentle vortexing or pipetting.

4.6. Briefly centrifuge the microcentrifuge tube to collect the liquid and beads, then place it back on the magnetic stand for 3 minutes or until the supernatant becomes clear. Carefully remove and discard the supernatant.

4.7. Add 200 µL of **Desulphonation Buffer** to the beads. Resuspend thoroughly by gentle vortexing or pipetting.

4.8. Incubate at room temperature for 10 minutes with continuous rotation. **A microtube rotator or mixer is strongly recommended for this incubation step.**

4.9. Briefly centrifuge the microcentrifuge tube to collect the liquid and beads, then place it back on the magnetic stand for 3 minutes or until the supernatant becomes clear. Carefully remove and discard the supernatant.

4.10. Add 400 µL of **Wash Buffer** to the beads. Resuspend thoroughly by gentle vortexing or pipetting.

4.11. Briefly centrifuge the microcentrifuge tube to collect the liquid and beads, then place it back on the magnetic stand for 3 minutes or until the supernatant becomes clear. Carefully remove and discard the supernatant.

4.12. Repeat Steps 4.10 and 4.11. After the final wash and discarding the supernatant, use a 10 µL tip to remove as much **Wash Buffer** as possible. This will help ensure efficient drying of the purification beads.

4.13. Place the **uncapped microcentrifuge tubes** on a heating element to **completely dry the Purification Beads**[10] at 55 °C for 5 - 10 minutes.

⚠ *[10]Note: **Ensure the Purification Beads are completely dry before proceeding to the elution step (Step 5)**. An example photo of dried beads is shown in the Supplementary Material (Section 7). If needed, extending the heating time may improve recovery of bisulfite-converted DNA. If the heating element has a lid, please keep the heating element lid open during this step. Because heating efficiency may vary between heating blocks, we recommend optimizing the heating time to achieve optimal recovery.*

5. Elution

5.1. Add 27 µL of **Elution Buffer**[11] directly to the dried beads and resuspend by pipetting or vortexing the microcentrifuge tube at low speed for 10 seconds.

⚠ *[11]Note: The volume of Elution Buffer can be adjusted according to the requirements of downstream applications. Varying the elution volume may affect elution efficiency: **approximately 5 µL of elution buffer typically remains associated with the magnetic beads after separation.***

5.2. Incubate the elution at 55°C for 4 minutes **with the cap closed**, mixing at 1000 RPM. Then place the microcentrifuge tube on a magnetic stand for 3 minutes or until the supernatant becomes clear.

5.3. Carefully transfer the supernatant (eluate) to a clean microcentrifuge tube or PCR tube for downstream amplifications.

Optional stopping point: The eluate, containing bisulfite-converted DNA, is immediately ready for downstream applications such as PCR analysis or next-generation sequencing. For storage, keep the eluate at -20 °C for short-term use or at -80 °C for long-term use.

6. Image of dried purification beads (Step 4.13)

Following incubation of beads at 55 °C for 5 - 10 minutes, sufficiently dried beads have matte, slightly cracked appearance.

7. Supplementary Material

1. Library Amplification with SuperMethyl™ dsDNA Library Kit for Illumina

In this section, you will find a supplemental library amplification protocol using **Ellis Bio SuperMethyl™ dsDNA Library Kit for Illumina (Ellis Bio)**. Please refer to the manufacturer's protocol for additional details.

1. Thaw all reagents on ice and mix thoroughly by gentle vortexing or pipetting.
2. Add nuclease-free water to the bisulfite-converted DNA eluted in Section 6 (Main protocol, Step 5) to bring the total volume to 22 µL.
3. In a PCR tube or plate, prepare the following amplification reaction mix on ice according to the **Protocol Table 6** below:

Protocol Table 6. PCR Amplification Reaction Setup[12]

Component	Volume
Bisulfite Converted DNA in water (from Step 2 above)	22 µL
2 x Uracil + PCR Mix 2.0	25 µL
P7 Index Primer (15 µM)	1.5 µL
P5 Index Primer (15 µM)	1.5 µL
Total Volume	50 µL

*[12]Note: *If index-free methylated adapters was used, use the index primers for PCR amplification.*

4. Gently mix the reaction by pipetting. Briefly centrifuge the tube to collect the liquid at the bottom of PCR tubes.
5. Place the reaction in a thermocycler and run the following program in **Protocol Table 7**:

Protocol Table 7. Thermocycler Program of PCR Amplification

Step	Temperature	Time	Cycles
Heated lid	105 °C	-	-
Initial Denaturation	98 °C	1 min	1
Denaturation	98 °C	10 sec	4 – 14 cycles[13]
Annealing	60 °C	30 sec	
Extension	72 °C	1 min	
Final Extension	72 °C	5 min	1
Hold	4 °C	Hold	1

[13]Note: Use 4–14 cycles depending on input amount and desired yield. Fewer cycles help preserve library complexity.

6. Purify the amplified library using 45 µL SuperMethyl™ DNA Selection Beads (Ellis Bio), or an alternative bead-based clean-up reagent, following the manufacturer's instructions .

2. Cell-Free DNA Sample Considerations

By running a fragment analysis (such as TapeStation or Bioanalyzer) on input cfDNA, one can determine if the majority of fragments are in the expected fragment range or if there is potential high molecular weight genomic DNA contamination. We recommend using size selection beads, such as SPRI beads to remove high molecular weight species above 700 bp. Follow the manufacturer's protocol for the size selection beads.