ELLIS BIO

SuperMethyl™-Fast Bisulfite Conversion Kit
50-Reactions, Magnetic Bead Purification

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

Product Highlights:

- ✓ **Ultra-Fast Bisulfite Conversion:** Achieve complete and ultra-fast bisulfite conversion in just 7 minutes—making it the fastest kit available for DNA bisulfite conversion.
- ✓ **High Efficiency:** Consistently delivers over 99.5% conversion of unmethylated cytosines, ensuring accurate analysis while preserving methylated cytosines.
- ✓ **Minimized DNA Damage:** Protects DNA integrity by significantly reducing degradation during the ultrafast conversion process.
- ✓ **Low Background Noise:** Reduces false positives and enhances the accuracy of 5mC signal detection.
- ✓ **Versatile Applicability:** Ideal for a wide range of applications, including PCR, methylation-specific PCR (MSP), arrays, library preparation, and next-generation sequencing (NGS).

This kit offers an unmatched combination of ultra-fast bisulfite conversion speed, accuracy, and reliability, setting a new benchmark in DNA methylation analysis.

Product Description

The **SuperMethyl™-Fast Bisulfite Conversion Kit** from Ellis Bio offers the revolutionary fastest and efficient bisulfite conversion solution available for DNA methylation analysis. Featuring a newly engineered, **ready-to-use fast bisulfite conversion reagent** and **a magnetic bead-based DNA purification method**, this kit streamlines the workflow by combining conversion and purification in one seamless process. Simply add the **SuperMethyl™-Fast Bisulfite Conversion Reagent** to your sample and incubate for **6-8 minutes at 98°C.**

Complete bisulfite conversion and DNA purification in **under 1 hour**, this kit not only delivers speed but also precision, achieving **over 99.5% conversion efficiency** while minimizing DNA degradation. Its versatility makes it indispensable for both research and clinical applications.

With an unmatched rapid workflow and exceptional performance, the **SuperMethyl™-Fast Bisulfite Conversion Kit** ensures reliable and reproducible results, making it the go-to choice for researchers who require both precision and quick turnaround times.

Number of tests per kit: 50 tests.

Your Kit includes:

Component	Volume and Quantity
SuperMethyl - Fast Conversion Reagent	1.5 mL x 7 vials
SuperMethyl - Fast Binding Buffer	28 mL
SuperMethyl - Fast Wash Buffer*	14 mL*
SuperMethyl - Fast Desulphonation Buffer	11 mL
SuperMethyl - Fast Elution Buffer	1.8 mL
SuperMethyl - Fast Purification Beads	0.55 mL

** SuperMethyl - Fast Wash Buffer requires the addition of 56 mL 100% ethanol (EtOH) before first use.*

User-supplied materials

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

Storage

The **SuperMethyl - Fast Conversion Reagent** can be stored at 4 - 25°C; we recommend 4°C storage. All other kit components can be stored at room temperature. The kit is stable for up to 6 months. Refer to the product label for the expiration date.

Applications

The kit is compatible with DNA from various sources including genomic DNA (gDNA) extracted from cells or tissues, gDNA from formalin-fixed paraffin-embedded (FFPE) samples, and cell-free DNA (cfDNA).

Input DNA amount needed

The kit requires an input DNA amount of 10 ng** to 2 µg. For optimal results, it is recommended to use 100 ng to 1 µg of DNA. We advise quantifying the DNA with a precise instrument such as a Qubit Fluorimeter (Thermo). Additionally, ensure that the DNA purity index (A260/A280) is between 1.7 and 1.9.

*** Users may be able to use inputs lower than 10 ng, as low as 200 pg. We recommend users run quality control tests on samples lower than 10 ng.*

Product Performance Indicators

- The C-to-T conversion rate at all unmethylated cytosines in both CpG and non-CpG contexts of λDNA exceeds 99.0%.
- The estimated methylation levels at all CpG sites in fully methylated pUC19 DNA is consistently above 95.0%.

CAUTION

This kit is for research use only. The **SuperMethyl - Fast Conversion Reagent**, **SuperMethyl - Fast Desulphonation Buffer**, and **SuperMethyl - Fast Wash Buffer** contains volatile ingredients. Cap the bottles tightly after use and store at recommended temperatures. Safety Data Sheets are available upon request.

Experimental Protocol

1. Reagent Preparation

Add 56 mL of 100% ethanol to the **SuperMethyl - Fast Wash Buffer** before the 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 **SuperMethyl - Fast Wash Buffer**.

2. Bisulfite Conversion

2.1. In a 1.5 mL nuclease-free microcentrifuge tube, pipette the volume to obtain 10 ng - 2 µg of input DNA. Add nuclease-free H_2O up to a total volume of 20 µL[1].

[1] Note: If the input DNA volume exceeds 20 µL, we recommend dividing the DNA into separate tubes for the downstream bisulfite conversion reactions and DNA binding on the beads. We also encourage users to perform additional testing as needed to optimize the protocol for their specific sample and workflow.

2.2. Add 180 µL **SuperMethyl - Fast Conversion Reagent**[2] and mix by pipetting. Aliquot the solution into PCR tubes with equal volumes (adjust volume based on thermocycler capacity).

Prepare the bisulfite conversion reaction following the instructions in the table below:

Component	Volume
Input DNA	20 µL (10 ng - 2 µg)
SuperMethyl - Fast Conversion Reagent	180 µL
Total Volume	200 µL

[2] Note: Before proceeding, please inspect the SuperMethyl - Fast Conversion Reagent vials for any signs of crystallization. Minor crystallization in the Fast Conversion Reagent is normal. If crystals are present, heat the vial at 60°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.

2.3. Mix thoroughly by vortexing for 5 seconds or pipetting. Briefly centrifuge the PCR tubes to avoid solution collection in the lids.

2.4. Place the capped PCR tubes in a thermal cycler and run the following program:

Temperature	Time
98 °C (with a 105 °C heated lid)	7 minutes[3]
4 °C	Hold

[3] Note: The 98 °C incubation time can be adjusted by the user from 6 to 8 minutes to optimize the cytosine-to-thymine (CT) conversion ratio (all exceeding 99.0%, with 7 minutes achieving over 99.5%). However, longer incubation may result in increased DNA damage and reduced DNA yield.

3. Purification and Storage

3.1. Add 500 µL of **SuperMethyl - Fast Binding Buffer** and 10 µL of **SuperMethyl - Fast Purification Beads**[4] to a 1.5 mL low-adhesion microcentrifuge tube.

*[4] Note: **SuperMethyl Magnetic Beads** settle quickly, so pipette or vortex well to ensure they remain suspended.*

3.2. Transfer the bisulfite converted reaction solutions from the PCR tubes into the 1.5 mL microcentrifuge tube containing the **SuperMethyl - Fast Binding Buffer** and **SuperMethyl - Fast Purification Beads**. 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. **(Optional: A microtube rotator can be used during this incubation.)**

3.3. Briefly centrifuge the microcentrifuge tube to collect the liquid and beads at the bottom, then place it on a magnetic stand for 5 minutes or until beads pellet has collected on magnet and the supernatant is clear. With the microcentrifuge tube on the magnetic stand, remove the supernatant and discard.[5]

[5] Note: Some beads may adhere to the sides of the well. Remove the supernatant slowly, allowing the beads to remain on the magnetic side as the liquid volume decreases.

3.4. Remove the microcentrifuge tubes from the magnetic stand for this step and each subsequent buffer addition. Add 400 µL of **SuperMethyl - Fast Wash Buffer** (ensure 100% ethanol was added in Step 1 before first use) to the beads and re-suspend by pipetting up and down or by vortexing the microcentrifuge tubes at low speed for 10 seconds. Briefly centrifuge the microcentrifuge tube to collect the liquid and beads, then place it back on the magnetic stand for 3 minutes. Carefully remove and discard the supernatant.

3.5. Add 200 µL of **SuperMethyl - Fast Desulphonation Buffer** to the beads. Re-suspend by pipetting up and down or by shaking the microcentrifuge tubes at low speed for 10 seconds. Allow the microcentrifuge tubes to stand at room temperature for 10 minutes. **(Optional: A microtube rotator can be used during this incubation.)** After incubation, briefly centrifuge the microcentrifuge tube to collect the liquid and beads, then place it back on the magnetic stand for 3 minutes. Carefully remove and discard the supernatant.

3.6. Add 400 µL of **SuperMethyl - Fast Wash Buffer** to the beads. Re-suspend by pipetting up and down or by shaking the microcentrifuge tubes at low speed for 10 seconds. Briefly centrifuge the microcentrifuge tube to collect the liquid and beads, then place it back on the magnetic stand for 3 minutes. Carefully remove and discard the supernatant.

3.7. Repeat Step 3.6. After the final wash, remove as much **SuperMethyl - Fast Wash Buffer** as possible to facilitate efficient drying of the beads.

3.8. Place the microcentrifuge tubes on a heating element to dry the beads at 55°C for 5 -10 minutes. Alternatively, the tubes can remain at room temperature for 10 - 30 minutes to dry the beads. Ensure that all residual **SuperMethyl - Fast Wash Buffer** is removed.

3.9. Add 10-30 µL of **SuperMethyl - Fast Elution Buffer**[6] directly to the dried beads and re-suspend by pipetting or shaking the microcentrifuge tubes at low speed for 10 seconds. Incubate at room temperature for 5 minutes, then place the microcentrifuge tubes on the magnetic stand for 1-3 minutes. Carefully remove the eluate (in the supernatant) and transfer it to a clean microcentrifuge tube.

*[6] Note: The volume of **SuperMethyl - Fast Elution Buffer** can be adjusted according to the requirements of downstream applications. Keep in mind that varying the elution volume may affect elution efficiency, with smaller volumes typically yielding more concentrated DNA but potentially lower overall recovery.*

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. The elution volume can be adjusted according to the specific requirements of your experiment, with smaller volumes yielding more concentrated DNA.