ELLIS BIO

# SuperMethyl™-Fast Bisulfite Conversion Kit
# 50-Reactions, Spin Column Purification

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

**Product Highlights:**

- ✓ **Ultra-Fast Bisulfite Conversion:** Achieve complete bisulfite conversion in just 8 minutes—making it the fastest kit available for comprehensive 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.
- ✓ **High DNA Recovery:** Achieves a DNA recovery rate of over 80%, ensuring that the majority of input DNA is retained for downstream applications.
- ✓ **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 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 spin-column DNA purification method**, this kit streamlines the workflow by combining conversion and purification in one seamless process. Simply add the reagent to your sample and incubate for less than **10 minutes at 98°C.**

Complete bisulfite conversion and DNA purification in **under 35 minutes**, 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 Buffer | 1.5 mL x 7 vials |
| SuperMethyl - Fast Binding Buffer | 28 mL |
| SuperMethyl - Fast Wash Buffer* | 7 mL* |
| SuperMethyl - Fast Desulphonation Buffer | 11 mL |
| SuperMethyl - Fast Elution Buffer | 1.5 mL |
| SuperMethyl Spin Columns & Collection Tubes | 50 pairs |

* SuperMethyl - Fast Wash Buffer requires the addition of 28 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.

**Storage**

The **SuperMethyl - Fast Conversion Buffer** 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 of unmethylated cytosines for input 200 ng λDNA is over 99%.

**CAUTION**
This kit is for research use only. The **SuperMethyl - Fast Conversion Buffer**, **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 28 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.*

2.2. Add 180 µL **SuperMethyl - Fast Conversion Buffer**[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 Buffer | 180 µL |
| **Total Volume** | 200 µL |

*[2]Note: Before proceeding, please inspect the SuperMethyl - Fast Conversion Buffer vial for any signs of buffer 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) | 8 minutes[3] |
| 4 °C | Hold |

*[3]Note: The 98 °C incubation time can be adjusted by the user from 6 to 10 minutes to optimize the cytosine-to-thymine (CT) conversion ratio (all exceeding 99.0%, with 8 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** to a **SuperMethyl Spin Columns**[4] on a provided Collection Tube.

*[4]Note: The collection tube can hold up to 800 µl when the column is inserted. To avoid*

*contamination of the column contents by the flow-through, be sure to empty the collection tube as needed before it reaches capacity.*

3.2. Transfer the bisulfite converted reaction solutions from the PCR tubes into the **SuperMethyl Spin Column** containing the **SuperMethyl Binding Buffer**. Close the cap and gently mix by inverting the column several times.

3.3. Centrifuge at 13,000 g for 30 - 60 seconds. Discard the flow-through.

3.4. Add 100 µL of **SuperMethyl - Fast Wash Buffer** to the spin column (ensure 100% ethanol was added before first use). Centrifuge at 13,000 g for 30 - 60 seconds. Discard the flow-through.

3.5. Add 200 µL of **SuperMethyl - Fast Desulphonation Buffer** to the spin column. Incubate at room temperature for 20 minutes. Centrifuge at 13,000 g for 30 - 60 seconds. Discard the flow-through.

3.6. Add 200 µL of **SuperMethyl - Fast Wash Buffer** to the spin column. Centrifuge at 13,000 g for 60 seconds. Discard the flow-through.

3.7. Repeat Step 3.6.

3.8. Transfer the spin column to a new nuclease-free 1.5 mL microcentrifuge tube.

3.9. Add 10-30 µL of **SuperMethyl - Fast Elution Buffer** to the center of the spin column membrane to elute the bisulfite-converted DNA[5]. Incubate at room temperature for 1 minute, then centrifuge at 13,000 x g for 1 minute.

*[5]Note: The eluate, containing bisulfite-converted DNA, is immediate 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.*

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