JBS Bisulfite DNA Conversion Kit
96 reactions

User manual
Cat. No. JBS-08878-MA

Version 1

Product Overview

This kit is designed for sodium bisulfite treatment of DNA including cell-free DNA followed by downstream clean-up using magnetic beads. The procedure consists of bisulfite (BSC) conversion, DNA binding, desulphonation, followed by DNA wash and elution to obtain high quality BSC-converted DNA suitable for downstream methylation DNA profiling.

Contents

Kit Components and Storage

JBS Bisulfite Conversion Kits are for research use only and are not intended for use in diagnostic procedures. All components are guaranteed for a shelf life of 12 months from the date of receipt when stored according to the table below.

Bisulfite DNA conversion kit (Kit size)	**Cat# BS-08878-MA** (96 rxns)	**Storage Temperature**
DNA binding beads (40 mg/ml)	1.1 ml	Room Temp.
Bisulfite conversion reagent*	1 tube (powder) x 10	Room Temp.
Buffer 1	12 ml	Room Temp.
Buffer 2	2.0 ml	Room Temp.
Buffer 3	2.2 ml	Room Temp.
Binding buffer	75 ml	Room Temp.
Wash buffer concentrate#	48 ml	Room Temp.
Desulphonation buffer	40 ml	Room Temp.
Elution buffer	75 ml	Room Temp.

*Prepare fresh bisulfite conversion reagent before each use, see “Before you begin”.

#Add 192 ml of 100% ethanol to 48 ml wash buffer concentrate to make wash buffer.

Notes Before Getting Started

- Use a new pipette tip after every dispense.
- Perform all procedures in a clean laboratory environment to avoid contamination,
- Kit has been validated with genomic and fragmented DNA.

Required Materials Not Provided

- 100% Ethanol
- Pipette tips and pipettes
- Instrument: vortex mixer, centrifuge, thermal cycler (≥100 µl capacity), heating block with shaking such as Eppendorf Thermomixer
- Magnetic stands to hold 1.5 ml tubes (Cat# JBS-Mag-1.5)
- Nuclease-free PCR tubes (0.2ml)

Procedure

Before you begin

Preparation of bisulfite conversion reagent:
The **Bisulfite conversion reagent** is supplied as a dry powder, prepare it for use as outlined below (each tube will yield enough reagent for 12 bisulfite conversion reactions):

1. Add 1048 µl **Buffer 1** and 136 µl **Buffer 2** to a tube of **Bisulfite conversion reagent.** Rotate at 20–30 rpm for 15 minutes to dissolve (alternatively vortex to dissolve), then add 186 µl **Buffer 3**, vortex to mix completely.
    a. **Note:** If the contents are difficult to dissolve, incubate in a 37°C water bath and vortex to ensure all solids are fully dissolved.
2. Once fully dissolved, incubate for 30 mins at room temperature. The **Bisulfite conversion reagent** is then ready for use.
    a. Note, use on the same day for optimal results. Any leftover reagent can be stored at 4°C, protected from light and used within a week. The **Bisulfite conversion reagent** must be brought to room temperature prior to use, and any precipitates must be dissolved by heating at 37°C and with vortexing.
3. Prepare wash buffer by adding 192 ml 100% ethanol to 48 ml **Wash buffer concentrate**, cap tightly and invert 10 times to mix well or swirl to mix. Mark the cap to indicate ethanol has been added and date the bottle.

1. For each bisulfite conversion reaction, place 5-50 ng DNA in a total volume of 17 µl in a 0.2ml PCR tube, add 3 µl **Buffer 2**.
2. Spin briefly, gentle pulse vortex or pipette up and down to mix, brief spin again.
3. Incubate for 15 min at 37°C in a thermocycler.
4. After incubation, add 130 µl of **Bisulfite conversion reagent** to the sample.
5. Mix well by pipetting or gentle pulse vortex, then centrifuge briefly to collect liquid.
6. Place the PCR tube in a thermal cycler and perform the following step:
    a. Incubate at 54°C for 16 hr, followed by incubation at 8°C until ready to proceed to the next step.
7. During incubation, Prepare **Binding buffer** with beads about 15–30 minutes before completion of bisulfite conversion reaction:
    a. Aliquot 600 µl of **Binding buffer** to a 1.5 ml tube.
    b. Add 10 µl of **DNA binding beads** to the tube.
    c. Note: DNA Binding beads settle very quickly, use P1000 to pipette beads up and down 10 times to resuspend beads before each addition of beads to ensure that beads are kept in suspension, then use P10 to pipette 10 ul beads to the blue sample tube.
8. Transfer the samples from the PCR tube into the 1.5 ml tubes containing the **Binding buffer** and beads.
9. Mix by pipetting up and down several times (or pulse vortex 5 times to mix).
10. Let sample tubes incubate at room temperature for 5 minutes (during the 5 min incubation, do a gentle pulse vortex to mix before starting the timer; after two minutes, do another gentle vortex; at the end of 5 min, do a final gentle vortex), then load the sample tubes in the sample rack.

11. Spin briefly, place the 1.5 ml tube on the 1.5 ml magnet for 3 minutes or until solution is clear of beads. Remove and discard the supernatant while keeping the tube on the magnet.
12. Remove tube from magnet and resuspend beads in 400 µl of **Wash buffer** by gentle vortex to resuspend beads completely, incubate for 30 s; do another gentle vortex and incubate for another 30 s.
13. Briefly spin down the tube and place on the magnet for 3 minutes or until solution is clear of beads. Remove and discard supernatant while keeping the tube on the magnet.
    a. Note, remove as much **Wash buffer** as possible. Use a P10 pipette to discard any remaining wash buffer (spin tube briefly to collect all residual wash buffer, put on magnet to separate, remove residual wash buffer with a P10 pipette).
14. Remove tube from magnet and resuspend beads in 200 µl of **Desulphonation buffer** by gentle vortex to resuspend beads completely.
15. Let tube stand at **room temperature (20-30°C) for 15 minutes**.
16. Briefly spin down the tube and place on the magnet for 3 minutes or until solution is clear of beads. Remove and discard supernatant while keeping the tube on the magnet.
    a. Note, DO NOT EXCEED a total incubation time of 20 mins with **Desulphonation buffer**. Adjust time as necessary to ensure that no sample remains in the **Desulphonation buffer** for more than 20 minutes.
17. Remove tube from magnet and resuspend beads in 400 µl of **Wash buffer** by gentle vortex to resuspend beads completely, incubate for 30 s; do another gentle vortex and incubate for another 30 s (beads may not resuspend after desulphonation buffer treatment, proceed as outlined).
18. Briefly spin down the tube and place on the magnet for 3 minutes or until solution is clear of beads. Remove and discard supernatant while keeping the tube on the magnet.
19. Repeat steps 17 to 18 for a total of two washes.
20. Briefly spin down tube to collect residual **Wash buffer** at the bottom of tube, then return tube to magnet for ~5 seconds, or as needed. Remove and discard the residual **Wash buffer** while keeping the tube on the magnet.
21. Dry the beads on magnet by opening tube cap at **room temperature** for about 5 - 10 minutes to remove residual **Wash buffer**.
    a. Note, beads will change in appearance from glossy black when still wet to a dull brown when fully dry. Do not allow the beads to become over-dried (dull brown with visible cracking).
22. Add ≥30 µl of **Elution buffer** kept at 55°C directly to the dried beads and resuspend beads completely by gentle vortex. Incubate at **55°C for 4 minutes with shaking at 1200 rpm using a thermomixer** (if thermomixer is not available, incubate at 55°C for 4 min, do a brief gentle vortex every minute)**.**
23. Brief spin to collect condensation and place the tube on the magnet for 1 minute or until solution is clear of beads.
24. Carefully transfer the eluted BSC-DNA to a new 1.5ml tube while keeping the tube on the magnet. Store BSC-DNA at -80°C or continue with downstream assays. It is recommended to aliquot out eluted DNA, for example 10 ul per aliquot.