JBS Bisulfite DNA Conversion Automation Kit 96 reactions

User manual
Cat. No. JBS-08878
For use with JPurX-S200 instrument

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 on the JPurX-S200 automation instrument. 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

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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 automation kit (Kit size)	**Cat# JBS-08878** (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.
Reagent Cartridge	96 pcs	Room Temp.
Sealing Tape	32 pcs	Room Temp.
Reaction Chamber	96 pcs	Room Temp.
Tip Holder	96 pcs	Room Temp.
Piercing Pin	100 pcs	Room Temp.
Small Tip	100 pcs	Room Temp.
Filter Tip	100 pcs	Room Temp.
Elution Tube	100 pcs	Room Temp.
Sample Tube	100 pcs	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

- Ensure the instrument is calibrated by performing piston testing.
- 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)
- Nuclease-free PCR tubes (0.2ml)

Procedure

For use with firmware version v1-201231-0 (file nameJBS-201231-0-new-rc) or later.

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.

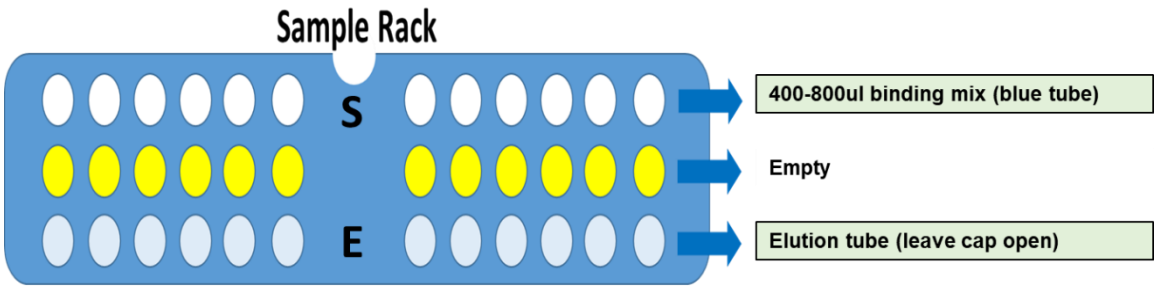
1. For each bisulfite conversion reaction, place 5-50 ng DNA in a total volume of 17 µl in a 0.2ml PCR tube, add 3 µl **Buffer 2**.
2. Spin briefly, gentle pulse vortex or pipette up and down to mix, brief spin again.
3. Incubate for 15 min at 37°C in a thermocycler.
4. After incubation, add 130 µl of **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 reagent cartridge according to **Appendix I**.
8. Prepare binding buffer with beads about 15–30 minutes before completion of bisulfite conversion reaction:
    a. Aliquot 600 µl of **Binding buffer** to each blue sample tube.
    b. Add 10 µl of **DNA binding beads t**o the blue sample 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.
9. Transfer the samples from the PCR tube into the blue sample tubes containing the **Binding Buffer** (from step 6) and beads.
10. Mix by gentle pulse vortex for 5 times.
    a. Note, ensure the total volume is no more than 800 µl.

11. 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.
12. Load elution tubes (1 per sample) with cap open to the sample rack.
13. Proceed to General instrument set-up with sample tube(s).

General Instrument Set-up

1. Turn on instrument to initialize.
2. Place **Reagent Cartridges**, reaction chambers, tip holder, piercing pins, and filter tips(s) in the instrument as instructed below using clean gloves or forceps. Refer to **Instrument Operation manual** for detailed set-up.

3. Load both the blue sample tube and the elution tube into the sample rack according to diagram below.



4. Press <Start>, the instrument will initiate.
5. After the initialization, enter in barcode # 08878.
6. Enter in the sample volume (400-900 µl) (i.e. 800 µl to account for overage).
7. Enter in the desired elution volume in a range of 30-100 µl (i.e. 050 µl).
8. Press **Start** to begin. Note, total processing time ~95 mins.
9. Transfer the eluted BSC-converted DNA into an appropriate cryogenic box and store at -80°C or continue with downstream assays. It is recommended to aliquot out eluted DNA, for example 10 ul per aliquot.

Appendix I

JPurX Barcode# 08878 cartridge layout

	Well	Reagent	ul/sample
Reagent Cartridge	10	Elution buffer	600
	9	-	-
	8	-	-
	7	-	-
	6	dH_20	600
	5	Wash buffer	600
	4	Wash buffer	600
	3	-	-
	2	Wash buffer	600
	1	Desulphonation buffer	300