

# Step 3: Protocol of sodium bisulfite conversion of DNA

## Protocol

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The procedure for bisulfite conversion of DNA is carried out with the EZ DNA Modification kit (Zymo Research). The kit can be stored at room temperature (15-25°C) for up to 12 months.

### Equipment and Reagents to Be Supplied by User:

- EZ DNA Methylation Kit (Zymo Research), catalog Nos. D5001 (50 rns.) & D5002 (200 rns.)
- Tube C (pink cap) of Urodiag<sup>®</sup> Multiplex PCR Kit (OncoDiag) = Methylated human control DNA
- Absolute ethanol (96-100%)
- 1.5 ml microcentrifuge tubes and *MicroAmp 8-Tube strip and 8-Cap Strip*
- Micropipets and Pipet tips with aerosol barrier
- Microcentrifuge (with rotor for 1.5 ml tubes)
- Vortexer
- Water bath or heating block (37°C and 50°C) or thermocycler (50°C)

### Buffer Preparation:

**Preparation of the conversion solution (CT-Conversion tube)** – The **CT Conversion** tube supplied is a solid mixture. The conversion solution must be prepared as follows:

1. Add 750  $\mu$ l sterile water and 210  $\mu$ l of **M-Dilution Buffer** to a tube of **CT Conversion** tube.
2. Mix at room temperature (15-25°C) with frequent vortexing for 5-10 minutes.

***Note:** It is normal to see trace amounts of undissolved reagent in the CT-Conversion tube. The CT-Conversion solution is designed for 10 separate reactions and must be used immediately following preparation (recommended) or stored for one week at +4°C and one month at -20°C.*

**Preparation of M-Wash Buffer**– Add 24 ml of ethanol to the 6 ml **M-Wash Buffer** concentrate (D5001) or 96 ml of ethanol to the 24 ml **M-Wash Buffer** concentrate (D5002) before use.

1. Add 5  $\mu$ l of M-Dilution Buffer to the DNA sample and adjust the final volume to 50  $\mu$ l with sterile water (see table below) in a 1.5 ml microcentrifuge tube.

	Tube C	Patient	
	Conc. 1.25 ng/ $\mu$ l	Conc. 1.25 ng/ $\mu$ l	Conc. 0.625-1.25 ng/ $\mu$ l
DNA sample	24 $\mu$ l	24 $\mu$ l	X $\mu$ l (15- 30 ng)
M-Dilution Buffer	5 $\mu$ l	5 $\mu$ l	5 $\mu$ l
Sterile water	21 $\mu$ l	21 $\mu$ l	X $\mu$ l (adjust the volume to 50 $\mu$ l)
Mix the sample by pipetting up and down.			

**Note: Optimal patient DNA input is 30 ng** (24  $\mu$ l of DNA sample at 1.25 ng/ $\mu$ l). DNA amount of 15-30 ng (0.625-1.25 ng/ $\mu$ l) can be also used.

2. Incubate the sample at 37°C for 15 minutes (water bath or heating block).
3. After the above incubation, centrifuge briefly the tube for 2 seconds to remove drops from the lid or sides.
4. Add 100  $\mu$ l of the conversion solution to DNA sample and mix by pipetting up and down 10 times.
5. Incubate the sample in the dark at 50°C for 15 hours and 30 minutes in water bath or heating block.
6. Centrifuge the tube for 2 seconds to remove drops from the lid or sides.
7. Incubate the sample at 4°C (e.g., on ice or fridge) for at least 10 minutes.

**Note: Alternative incubation condition.** For steps 5 & 6 of the protocol, transfer the sample in a 0.2 ml tube (e.g., MicroAmp 8-Tube strip and 8-Cap Strip, applied Biosystems).

Incubate the sample in a thermocycler at 50°C for 15 hours and 30 minutes, then « hold » at 4°C.

8. Add 400  $\mu$ l of **M-Binding Buffer** to a **Zymo-Spin IC Column** and place the column into a provided **Collection tube**.
9. Load the sample (~150  $\mu$ l) into the Zymo-Spin IC Column containing the M-Binding Buffer. Close the column and mix by inverting the column at least 5 times.
10. Centrifuge the column at full speed (14,000 rpm, 16,800 rcf) for 30 seconds. Discard the flow-through.
11. Add 100  $\mu$ l of **M-Wash Buffer** to the column. Close the column and centrifuge at full speed for 30 seconds.
12. Add 200  $\mu$ l of **M-Desulphonation Buffer** to the column and let stand at room temperature for 20 minutes. After the incubation, centrifuge the column at full speed for 30 seconds.
13. Add 200  $\mu$ l of **M-Wash Buffer** to the column. Close the column and centrifuge at full speed for 30 seconds. Add another 200  $\mu$ l of **M-Wash Buffer** to the column and centrifuge at full speed for an additional 30 seconds.
14. Place the column into a 1.5 ml microcentrifuge tube and centrifuge at full speed for 30 seconds to eliminate possible traces of M-Wash Buffer, which will be removed by pipetting.
15. Add 10  $\mu$ l of **M-Elution Buffer** directly to the column matrix, and let stand at room temperature for 5 minutes. Centrifuge the microcentrifuge tube at full speed for 30 seconds to elute the bisulfite modified DNA.

The bisulfite-converted DNA solution is ready for immediately analysis or can be stored at -20°C for later use.