DNA Methylation Bisulfite Kit

RK30203

Instruction Manual

Version:N18E14V1.0

Contents

1. Introduction

The DNA Methylation Bisulfite Kit combines DNA denaturation and bisulfite conversion in one step and includes an additional heating step to promote rapid DNA denaturation. This product is a ready-to-use conversion reagent. Simply add this reagent to the DNA sample, and the conversion can be completed within 40 minutes. Subsequently, use the unique low-elution spin columns for desulfonation and purification of the converted DNA. The kit can accommodate conversion of samples ranging from 100 pg to 2 μg, with a conversion rate of unmethylated cytosine to uracil ≥99%. The high-yield converted DNA is highly suitable for applications such as PCR, qPCR and next-generation sequencing.

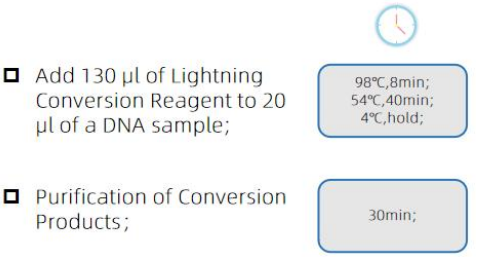
2. Components

Components	Component number	10 RXN	50 RXN	200 RXN
Conversion reagent	RM30451	1.3 mL	1.3 mL*5	1.3 mL*20
M-Binding buffer	RM30445	6 mL	30 mL	125 mL
M-Wash buffer	RM30446	1.2 mL	6 mL	24 mL
M-Desulphonation buffer	RM30447	2 mL	10 mL	40 mL
M-Elution buffer	RM30448	200 μL	1 mL	4 mL
Spin-column	RM30449	10 pk	50 pk	200 pk
Collection tube	RM30450	10 pk	50 pk	200 pk

3. Storage and Transport Conditions

Transport and store at room temperature.

4. Procedure



4.1. Buffer Preparation

4.1.1. Before the first use, ethanol should be added to the M-Wash Buffer according to the instructions on the reagent bottle label. Check the box to mark it to avoid multiple additions. Tighten the bottle to prevent ethanol evaporation.

10 RXN: Add 4.8 mL of ethanol to 1.2 mL of M-Wash Buffer;

50 RXN: Add 24 mL of ethanol to 6 mL of M-Wash Buffer;

200 RXN: Add 96 mL of ethanol to 24 mL of M-Wash Buffer.

4.2. DNA denaturation and bisulfite conversion processes

4.2.1. In a PCR tube, add 130 µl of Conversion Reagent to a 20 µl of DNA sample. Mix well, then centrifuge quickly to ensure no droplets in the cap or sides of the tube.

Note: If the volume of DNA is less than 20 µl, compensate with water. Samples >20 µl must be processed using multiple conversion reactions. Replicate reactions can be cleaned using the same column.

4.2.2. Place the PCR tube in a thermal cycler and perform the following steps:

	Temperature	Time
	98℃	8 min
	54℃	40 min
	4℃	Hold

Note: Store at 4°C for up to 20 hours. The 4°C storage step is optional.

4.3. Purification

4.3.1. Add 600 µl of M-Binding Buffer to a Spin-Column and place the column into a Collection Tube.

Note: The capacity of the collection tube with the column inserted is 800 µl. Empty the collection tube whenever necessary to prevent contamination of the column contents by the flow-through.

4.3.2. Load the sample (from Step 4.2.2) into the Spin-Column containing the M-Binding Buffer. Close the cap and mix by inverting the column several times.

4.3.3. Centrifuge at full speed (12,000 x g) for 30 seconds. Discard the flow-through.

4.3.4. Add 100 µl of M-Wash Buffer to the column. Centrifuge at full speed (12,000 x g) for 30 seconds.

4.3.5. Add 200 µl of M-Desulphonation Buffer to the column and let stand at room temperature (20-30°C) for 15-20 minutes. After the incubation, centrifuge at full speed (12,000 x g) for 30 seconds.

4.3.6. Add 200 µl of M-Wash Buffer to the column. Centrifuge at full speed (12,000 x g) for 30 seconds. Repeat this wash step.

4.3.7. Repeat step4.3.6.

4.3.8. Discard the flow-through. Place the column into a 1.5 ml microcentrifuge tube and add 10 µl of M-Elution Buffer directly to the column matrix. Centrifuge for 30 seconds at full speed (12,000 x g) to elute the DNA.

Note: Alternatively, water or TE (pH ≥ 6.0) can be used for elution if required for your experiments. The DNA is ready for immediate analysis or store at or below -70°C for long term storage. The elution volume can be > 10 µl depending on the requirements of your experiments, but small elution volumes will yield higher DNA concentrations.

ABclonal Biotechnology Co. Ltd.

E-mail:info@abclonal.com

Web:www.abclonal.com

Headquarters: Building 5, Precision Medicine Industry Base Project I, Gaokeyuan 3rd Road,Donghu New Technology Development Zone, Jiangxia District, Wuhan, Hubei, China