TIANGEN®

DNA Bisulfite Conversion Kit

For complete bisulfite conversion/ cleanup of DNA

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DNA Bisulfite Conversion Kit

(Spin Column)

Cat.no. GDP215

Kit Contents

	Contents	GDP215-02 50 preps
GDP215H	Bisulfite Mix	10 preps/tube × 5
	Buffer BM	5 ml
	Buffer DB	10 ml
	Buffer BL	30 ml
	Buffer PB	30 ml
	Buffer PW	2 × 15 ml
	Carrier RNA	310 μg
	RNase-Free ddH_2O	1 ml
	Buffer EB	15 ml
	Spin Columns CB1	50
	Collection Tubes 2 ml	50
	Handbook	1
GRK196	Buffer DP	0.5 ml

Note: GDP215H, GRK196 are shipped and packaged separately

Storage

DNA Bisulfite Conversion Kit should be kept in dry place and can be stored at room temperature (15-30°C) for up to 15 months. If any precipitate has been formed in buffers, please place the buffer at room temperature or warm it at 37°C for 10 min to dissolve the precipitate. Buffer DP should be kept at -30~-15°C for up to 18 months. After the addition of ethanol (96-100%), Buffer DB should be stored at 2-8°C, Buffer DP and stock solution of Carrier RNA should be stored at -30~-15°C.

Introduction

Epigenetics is the study of heritable changes in gene expression and regulation that are not caused by changes in nucleic acid sequence. Although there are various epigenetic effects such as RNA interfere (gene silencing) and histone modification, DNA methylation is the most crucial epigenetic mechanism in eukaryotes.

This kit is designed to study the bisulfite conversion in the DNA methylation process. The whole experimental process could be finished within 2 h, and the conversion rate from unmethylated Cytosine residues to Uracil can be higher than 99%. Meanwhile, this kit uses unique DNA protection components which can guarantee the yield and purity by preventing DNA fragmentation. In addition, this kit also uses spin column to get rid of sulphurous acid residues to make the experimental process quick and easy.

Product Features

Simple and Fast: The process of conversion and purification can be finished within 2 h. This experiment has low requirement on lab equipment, which is good for the study of bisulfite conversion of DNA in different research organization and institution.

High Conversion Rate: The conversion rate from unmethylated Cytosine residues to Uracil can be higher than 99%.

High Sensitivity: 500 pg - 2.5 μg of DNA sample can be processed by this kit.

High Performance: DNA product purified by this kit works well as template in methylation-specific PCR, and it is also suitable for other methylation study methods like sequencing and biochip.

Important Notes

1. Add 60 ml ethanol (96-100%) to 15 ml Buffer PW and add 30 ml ethanol (96-100%) to 10 ml Buffer DB before use.
2. If the amount of DNA sample is less than 100 ng, we strongly recommend adding Carrier RNA to Buffer PB until the DNA concentration reach 10 μg/ml.
3. All centrifugation steps should be carried out in a conventional table-

top microcentrifuge at room temperature (15-30°C).

4. Spin columns should be equilibrated with Buffer BL before use to activate the silica membrane to increase the yield.

Protocol

Bisulfite Conversion

1. Thaw the genomic DNA sample.
2. Prepare Bisulfite Mix according to specific experimental requirements. 850 µl of Buffer BM should be added to each tube of Bisulfite Mix powder. Mix by vortex until all the powders are dissolved. This step will cost 5 min.
   Note: The volume of dissolved Bisulfite Mix is around 950 µl, which can process 10 samples. Dissolved Bisulfite Mix should be stored at -20°C for up to 1 month.
3. According to Table 1, set up the Bisulfite Conversion Reaction in a 200 µl centrifuge tube.

Table 1 Bisulfite Conversion Reaction System

Components	Volume
DNA Sample	X µl (the optimal DNA amount is 500-1000 ng)
ddH_2O	20-X µl (the volume of DNA and ddH_2O should be no more than 20 µl)
Buffer DP	10 µl
Bisulfite Mix	90 µl
Total	120 µl

4. After the reaction setup, according to Table 2, start the bisulfite conversion reaction on a thermal control system like PCR instrument. The whole process will cost 1 h.

Table 2 Bisulfite Conversion Reaction Thermal Cycle Condition

Temperature	Time
95°C	10 min
64°C	30-60 min
4°C	Hold

Note: If the DNA sample is less than 500 ng at beginning, incubate at 64°C for 30 min would be enough. If the DNA sample is more than 500 ng, incubate at 64°C for 60 min. In addition, in the process of reaction, ensure that the cap of tube is closed, and heat the lid.

DNA Purification after Bisulfite Conversion

5. Column equilibration: Add 500 µl of Buffer BL to a Spin Column CB1 (place the column into a collection tube), centrifuge for 1 min at 12,000 rpm (~13,400 × g). Discard the flow-through, place the column back into the same collection tube **(Please use the column treated in the same day)**.
6. After the Bisulfite Conversion Reaction, transfer the reaction mix to a clean 1.5 ml microcentrifuge tube by briefly centrifuge.
7. Add 5 times volume (600 µl) of Buffer PB to the 1.5 ml microcentrifuge tube and mix.
8. Transfer the solution from step 7 to the Spin Column CB1 (place the column in a collection tube), incubate at room temperature for 2 min, centrifuge at 12,000 rpm (~13,400 × g) for 30-60 sec. Discard flow-through and place the Spin Column CB1 back into the same collection tube.

   Note: The capacity of Spin Column CB1 is 800 µl. If the volume of sample is more than 800 µl, please centrifuge the sample separately.
9. Add 600 µl Buffer PW (Ensure that ethanol (96-100%) has been added before use) to the Spin Column CB1 and centrifuge at 12,000 rpm (~13,400 × g) for 30-60 sec. Discard the flow-through and place the Spin Column CB1 back into the same collection tube.
10. Add 600 µl Buffer DB to the Spin Column CB1, incubate at room temperature (15-30°C) for 15 min, centrifuge at 12,000 rpm (~13,400 × g) for 30-60 sec. Discard the flow-through and place the Spin Column CB1 back into the same collection tube.
11. Add 600 µl Buffer PW to the Spin Column CB1 and centrifuge at 12,000 rpm (~13,400 × g) for 30-60 sec. Discard the flow-through.
12. Repeat step 11.
13. Place the Spin Column CB1 back into the same collection tube, centrifuge at 12,000 rpm (~13,400 × g) for 2 min to completely remove the residual buffer in the Spin Column CB1. Place the column at room temperature for several minutes to dry the membrane to avoid the side effect of residual buffer to subsequent experiments.

    Note: Residual ethanol will influence the subsequent PCR experiments.

14. Place the Spin Column CB1 into a clean microcentrifuge tube and add 20 µl Buffer EB to the center of the membrane and incubate 2 min at room temperature (15-30°C). Centrifuge at 12,000 rpm (~13,400 × g) for 2 min and collect the flow-through.

    Note: The volume of elution buffer should not be less than 20 µl, or else it may affect recovery efficiency. Transfer the flow-through solution back to the column and repeat step 14 would increase DNA yield. If the purified genomic DNA would be used within 24 h, please store the DNA at 4°C; for longer storage, genomic DNA should be stored at -20°C. In addition, in the subsequent PCR amplification reaction, we recommend adding 2 µl of genomic DNA as template in a 20 µl PCR reaction.

Analysis of yield and purity of DNA

After the Bisulfite Conversion, in the genomic DNA, unmethylated "C" will be converted to "U", so the purified genomic DNA will composed mostly by "A", "T" and "U". The original pattern of base pairing will no longer exist, instead of which single strand DNA and non-specific pairing will be the most common pattern of nucleic acid. The absorption of this kind of nucleic acid at OD_{260} is close to RNA. So an OD_{260} of 1 corresponds to a 40 ng/ul purified genomic DNA solution.

In addition, since this specificity of purified genomic DNA, the OD_{230} value would be abnormal, which may lead to an unstable OD_{260}/OD_{230} value. Previous study showed that this won't affect the subsequent PCR reaction.

OD_{260}/OD_{280} value should be within 1.7-1.9. If distilled water was used to elute DNA, OD_{260}/OD_{280} value would be lower, but it does not mean that the purity of genomic DNA is lower since the pH value and ions would affect the light absorption value.