MANUAL

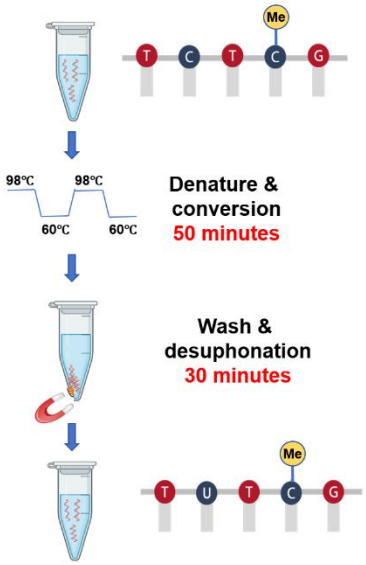
DNA Bisulfite Conversion Kit (Magnetic Bead Method)

Catalog #: NAE-BIS-MAG

ISO 13485:2016

Purpose

This kit is used to convert unmethylated cytosine residues into uracil for gene methylation analysis in genomic DNA. Methylated cytosine residues (5-methylcytosine) remain unchanged. The converted DNA is ideal for PCR, MSP, MSRE, pyrosequencing and Next-Gene sequencing. The schematic procedure of DNA Bisulfite Conversion Kit (Magnetic Bead Method) was as follows:



Kit Components (50 sample kit)

Store kit in a dry environment at room temperature for up to 24 months. Kit must be used within the validity period to ensure correct performance.

Component	Catalogue #	Size	Quantity
Transformation Reagent	NAE-TR	1.5 mL/vial	4 vials
Magnetic Bead Solution B	NAE-MAG-B	0.6 mL/vial	1 vial
Binding Buffer B	NAE-BB-B	30 mL/bottle	1 bottle
Wash Buffer B	NAE-WB-B	30 mL/bottle	2 bottles
Desulphonation Buffer	NAE-DS	20 mL/bottle	1 bottle
Elution Buffer A	NAE-EB-A	1.5 mL/vial	3 vials

Note: Do not mix reagents from different lots.

Required Materials (NOT INCLUDED)

1. Magnetic frame
2. Tabletop Centrifuge
3. Vortex Mixer
4. Water bath or dry heat block
5. Thermocycler
6. Pipettes and sterile nuclease free pipette tips (barrier tips recommended)
7. Nuclease free microfuge, conical, and PCR tubes

Sample Requirements

1. Sample type: Human genomic DNA. The amount of DNA for each modification can be 2 ng-1 µg. For optimal modification, the input DNA amount should be 100-500 ng.

Protocol

1. **Sample preparation**: Thaw genomic DNA samples on ice for 10-30 minutes immediately prior to use.
2. **Bisulfite Conversion**:

   2.1 Prepare the bisulfite conversion reaction(s) in a PCR tube(s) as shown in **Table 1**.

Table 1. Bisulfite Conversion System

Component	Volume/reaction
DNA Sample*	40 µL
Transformation Reagent	110 µL
Total	150 µL

***Note**: If DNA sample is less than 40 µL, add distilled water to bring total volume to 40 µL.*

***Note**: If required for your thermocycler, individual bisulfite conversion reactions can be split between two labeled tubes and then recombined afterwards.*

2.2 Set cycle conditions on PCR thermocycler according to **Table 2**.

Table 2. Thermocycler Cycling Conditions for Bisulfite Treatment

Temperature	Duration
98°C	5 minutes
60°C	20 minutes
98°C	5 minutes
60°C	20 minutes

a. Proceed immediately to step 3 (DNA Extraction).

3. **DNA Extraction**

3.1 Nucleic acid binding:

3.1.1 Add 600 µL of Binding Buffer B and 10 µL Magnetic Bead Solution B to a labeled 1.5 mL microcentrifuge tube.

3.1.2 Add transformed DNA (from step 2), vortex briefly, and incubate at room temperature for 5 minutes.

3.1.3 Centrifuge briefly at low speed and place on a magnetic rack for 2 minutes. Discard clarified waste liquid waste.

3.1.4 Add 400 µL Wash Buffer B, vortex, and centrifuge briefly at low speed. Place on magnetic rack for 2 minutes. Discard clarified liquid waste.

3.1.5 Add 400 µL Desulphonation Buffer and vortex briefly. Incubate at room temperature for 15 minutes.

3.1.6 Centrifuge briefly at low speed. Place on magnetic rack for 2 minutes. Discard clarified liquid waste.

3.1.7 Add 400 µl Wash Buffer B, vortex, and centrifuge briefly at low speed. Place on magnetic rack for 2 minutes. Discard clarified liquid waste.

3.1.8 Repeat previous wash, step 3.1.7.

3.1.9 Open tube in clean environment and dry at room temperature for 2 minutes.

***Note**: We recommend doing this step in a clean, sterilized PCR hood.*

3.2 Nucleic acid elution:

3.2.1 Add 30 – 50 µL Elution Buffer A and vortex briefly. Incubate at 56°C for 3 minutes. Vortex thoroughly for 5 seconds every minute.

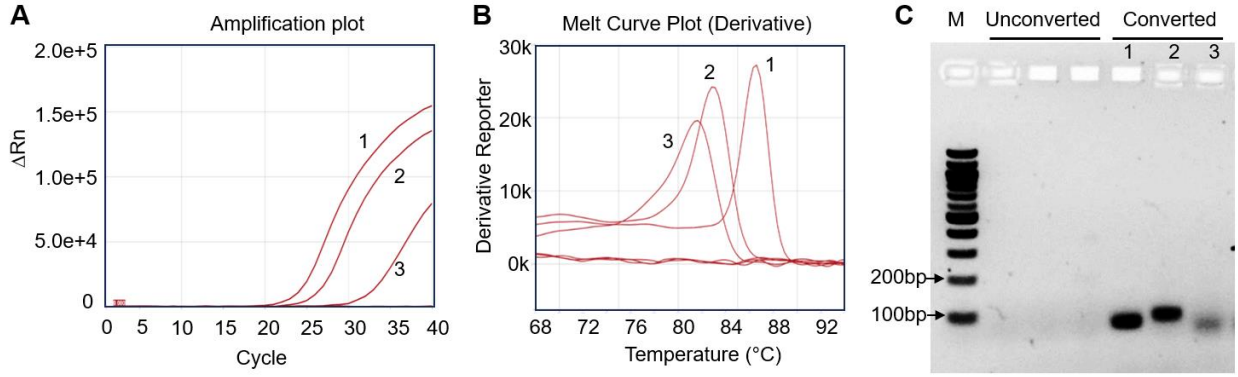
3.2.2 Place on magnetic rack for 2 minutes. Transfer clarified liquid to a new, labeled centrifuge tube.

***Note:** The clarified liquid contains the extracted nucleic acid. Do not discard!*

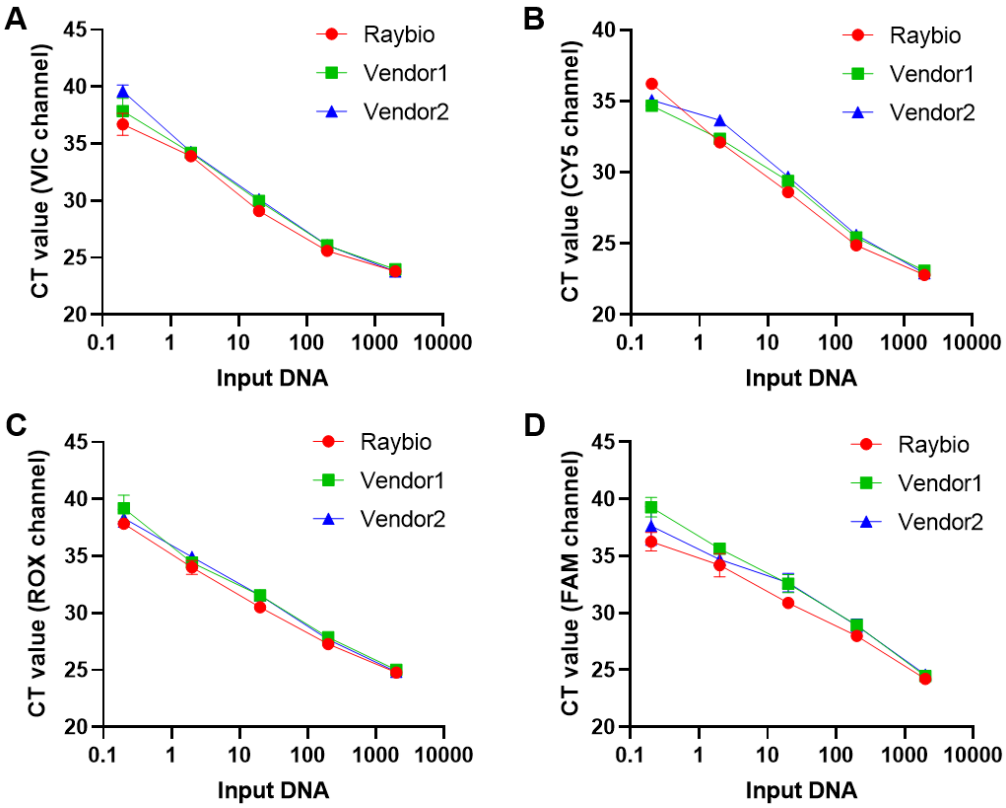
3.2.3 Purified DNA can be used immediately or stored below -20°C. For long term storage, store at or below -70°C.

Product Performance

Complete cytosine conversion: 200ng of genomic DNA isolated from A549 cells was treated with or without the DNA Bisulfite Conversion Kit (Magnetic Bead Method). The converted DNA and unconverted DNA were amplified with three specific primers by SYBR-green real time PCR method. The amplification plot (A), melt curve plot (B) and agarose gel staining (C) assay was determined after 40 cycles of real time PCR, respectively.



Effective DNA methylation conversion: Fully methylated human genomic DNA at various amounts (0.2ng-2000ng) were converted using the DNA Bisulfite Conversion Kit from RayBiotech and two vendors. The detection was performed by TaqMan qPCR method with four channels (A-D).



Precision: Intra-assay (Within-Run) of DNA methylation conversion was performed. 200ng genomic DNA isolated from RKO cells were tested in 8 separate assays by TaqMan qPCR assay. Precision testing showed that the coefficient of variation (CV) of the precision Ct (cycle threshold) values within lots is less than 0.75%.

Vendors	**Raybiotech**				**Vendor 1**			
Channel	**VIC**	**FAM**	**ROX**	**CY5**	**VIC**	**FAM**	**ROX**	**CY5**
Repeat 1	25.72	26.80	26.93	23.98	25.81	27.27	26.94	24.29
Repeat 2	25.71	26.87	26.93	23.95	25.75	27.45	26.93	24.22
Repeat 3	25.83	27.23	27.14	24.15	25.68	27.55	26.75	24.45
Repeat 4	25.70	26.69	26.86	23.98	25.72	27.42	26.91	24.13
Repeat 5	25.84	26.84	27.04	23.99	25.72	27.24	26.89	24.16
Repeat 6	25.93	27.02	27.18	24.18	25.70	27.55	26.96	24.26
Repeat 7	25.89	27.20	27.12	24.11	25.54	26.59	26.75	23.96
Repeat 8	25.77	27.16	27.07	24.22	25.58	26.72	26.76	23.98
Average	**25.80**	**26.98**	**27.03**	**24.07**	**25.69**	**27.22**	**26.86**	**24.18**
SD	**0.09**	**0.20**	**0.12**	**0.11**	**0.09**	**0.37**	**0.09**	**0.16**
CV%	**0.34**	**0.75**	**0.43**	**0.45**	**0.35**	**1.36**	**0.34**	**0.67**

Example 1 of test results: Cell free DNAs were isolated from 5 cases of human plasma (2ml) and converted by kits from RayBiotech and two vendors. TaqMan qPCR was performed using 4 channels.

Vendors	**Raybiotech**				**Vendor 1**				**Vendor 2**			
DNA-sample	VIC	FAM	ROX	CY5	VIC	FAM	ROX	CY5	VIC	FAM	ROX	CY5
cf DNA 1	30.23	N/A	31.78	28.85	30.53	N/A	32.04	29.26	30.39	N/A	32.91	29.45
cf DNA 2	31.56	N/A	32.74	30.01	32.00	N/A	33.69	30.31	32.25	N/A	34.50	31.82
cf DNA 3	34.30	36.52	38.05	31.21	35.24	41.02	36.22	31.20	34.95	38.98	36.82	32.30
cf DNA 4	34.62	37.72	38.19	30.57	37.45	N/A	38.36	31.42	34.80	38.88	37.59	31.64
cf DNA 5	32.82	35.78	36.96	29.62	34.37	37.93	36.30	30.55	34.99	37.51	37.41	30.50

Example 2 of test results: Stool DNAs were isolated from 5 cases of human and converted by kits from RayBiotech and two vendors. TaqMan qPCR was performed using 4 channels.

Vendors	Raybiotech				Vendor 1				Vendor 2			
DNA-sample	VIC	FAM	ROX	CY5	VIC	FAM	ROX	CY5	VIC	FAM	ROX	CY5
Stool DNA 1	32.21	27.3	34.23	28.42	32.22	30.66	35.53	29.48	32.66	26.95	35.43	29.38
Stool DNA 2	31.61	28.4	34.08	28.64	32.88	29.52	35.43	29.38	32.55	26.52	34.23	29.21
Stool DNA 3	33.1	26.56	35.94	29.86	33.24	27.71	36.4	30.42	33.58	24.96	36.34	30.84
Stool DNA 4	32.75	27.9	34.27	28.89	32.93	25.93	34.43	29.2	32.62	24.73	34.52	29.41
Stool DNA 5	34.16	27.86	35.19	32.17	34.38	24.85	N/A	32.01	N/A	23.84	34.7	31.3

References

1. Clark, Susan J *et al*. DNA methylation: Bisulphite modification and analysis. Nature Protocols, London1.5 (Dec 2006): 2353-64.
2. Hikoya Hayatsu. Bisulfite Modification of Nucleic Acids and their Constituents. Progress in Nucleic Acid Research and Molecular Biology, 1976, 16, 75-124.
3. Richard Y-H. Wang *et al*. Comparison of bisulfite modification of 5-methyldeoxycytidine and deoxycytidine residues. Nucleic Acids Research, 1980, 8(20), 4777-4790.