ab117127 – Fast Bisulfite Conversion Kit

Instructions for Use

For bisulfite conversion directly on a DNA sample

This product is for research use only and is not intended for diagnostic use.

Version 1a Last Updated 4 February 2019

Table of Contents

1. BACKGROUND

DNA methylation occurs by the covalent addition of a methyl group at the 5-carbon of the cytosine ring, resulting in 5-methylcytosine. There are various methods used to assess DNA methylation states. However, only bisulfite modification of genomic DNA, followed by PCR amplification, cloning, and sequencing of individual PCR amplimers yields reliable information on the methylation states of individual cytosines on individual DNA molecules. By treating DNA with bisulfite, cytosine residues are deaminated to uracil while leaving 5-methylcytosine intact:

	Unmethylated DNA	**Methylated DNA**
Original Sequence	C-C-G-T-C-G-A-C-G-T	C-^{M}C-G-T-^{M}C-G-A-^{M}C-G-T
After Bisulfite Conversion	U-U-G-T-U-G-A-U-G-T	U-^{M}C-G-T-^{M}C-G-A-^{M}C-G-T

The traditional bisulfite conversion method needs 12-16 h for bisulfite treatment, resulting in heavy DNA degradation (>80%), high inappropriate methylcytosine deamination (>3.5%) and low cytosine conversion rate (<95%). In March 2005 a fast DNA bisulfite modification method was developed to overcome these problems – shortening the entire bisulfite process from 16 hours to just 1.5 hours, significantly improving cytosine conversion efficiency (>99.9%), and effectively preventing converted DNA degradation.

To effectively and efficiently prepare converted DNA for use in various downstream analyses, an ideal DNA bisulfite modification method should be (1) highly accurate to allow for the complete conversion of cytosine to uracil (correct conversion) without deamination of methylcytosine to thymine (inappropriate conversion); and (2) rapid enough to enable the bisulfite process to be as short as possible, as rapid DNA methylation analysis is highly demanded for basic research and particularly for clinical applications.

Abcam's Fast Bisulfite Conversion Kit perfects DNA bisulfite treatment for better DNA methylation analysis. This kit greatly improves the currently used methods/kits for DNA bisulfite modification. With the novel and optimized bisulfite composition, ab117127 allows for the DNA modification step to be

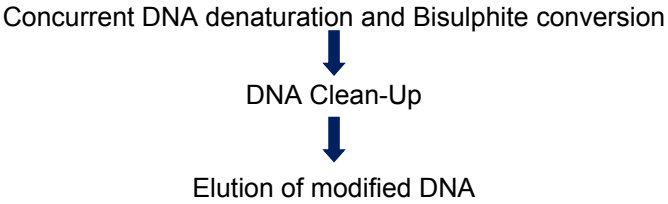
just 20 minutes with a complete cytosine conversion. More importantly, it greatly reduces inappropriate conversion of 5-methylcytosine to thymine (<0.1%). ab117127 is suitable for MS-PCR, real time MS-PCR, and methylation microarray.

The kit has the following advantages and features:

- Convenient single temperature incubation without the need for a separate DNA denaturation step.
- The fastest and most convenient protocol that can be finished in as short as 30 minutes.
- Completely converts unmethylated cytosine into uracil (>99.99%) with negligible inappropriate or error conversion of methylcytosine to thymine (<0.1%).
- Powerful protection against DNA degradation, with over 90% of DNA loss prevented.
- Extremely low requirement of input DNA for modification - only 0.2 ng or 50 cells.
- Simple, reliable, and consistent modification conditions

As a next generation bisulfite conversion tool, ab117127 contains all reagents required for an ultra-fast bisulfite conversion on a DNA sample. With the unique conversion mix solution which contains powerful DNA protection reagents, DNA denaturation status is sustained throughout the entire bisulfite DNA conversion process, thereby enabling 100% of DNA to be modified in single stranded form without chemical and thermophilic degradation. Thus, this novel approach leads to an accelerated conversion of all cytosine to uracil with negligible methylcytosine deamination. The non-toxic DNA capture solution enables DNA to tightly bind to the column filter, thus DNA cleaning can be carried out on the column to effectively remove residual bisulfite and salts.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit as given in the table upon receipt away from Light.

Observe the storage conditions for individual prepared components in sections 9 & 10.

For maximum recovery of the products, centrifuge the original vial prior to opening the cap.

Check if there are any precipitates in the bottle of Conversion Mix Solution prior to use. If so, shake the bottle to re-dissolve it. Tightly cap after each opening or usage.

5. MATERIALS SUPPLIED

Item	50 Tests	Storage Condition (Before Preparation)
Conversion Mix Solution	6 mL	RT
Capture Solution	15 mL	RT
Desulphonation Solution	60 µL	RT
Elution Solution	1 mL	RT
Conversion Enhancer	5 vials	RT
Denaturation Enhancer	600 µL	RT
F-Spin Column	50	RT
F-Collection Tube	50	RT

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Adjustable pipette or multiple-channel pipette
- Multiple-channel pipette reservoirs
- Thermocycler with heated lid*
- 0.2 mL PCR tubes
- 1.5 mL microcentrifuge tubes
- 100% Ethanol
- 90% Ethanol
- Desktop centrifuge (up to 14,000 rpm)

**Since the bisulfite reaction is not overlaid with mineral oil, only thermal cyclers with heated lids are suitable for this procedure*

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not use kit or components if it has exceeded the expiration date on the kit labels
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- **This kit is sold based on number of Reaction. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

9.1 Conversion/Denaturation Solution

Add 1 mL of Conversion Mix Solution to 1 vial of Conversion Enhancer. Mix by inverting and shaking the vial repeatedly for 2 min. Add 80 µL of Denaturation Enhancer to the vial and mix by inverting and shaking for an additional 3 min (trace amount of undissolved Conversion Enhancer may remain, which is normal as Conversion Enhancer is saturated in solution).

Prepared Conversion/Denaturation Solution can be stored at room temperature for up to 2 weeks without significant loss of efficiency. For the best results, the prepared solution should be used immediately.

9.2 90% Ethanol

Add 1 mL of distilled water to 9 mL of Ethanol.

9.3 Final Desulphonation Buffer

Add 12 µL of Desulphonation Solution to every 1 mL of 90% Ethanol and mix.

10. SAMPLE PREPARATION

10.1 Input DNA Amount:

DNA amount can range from 200 pg to 1 µg per reaction. An optimal amount is 50-200 ng per reaction. Starting DNA may be in water or in a buffer such as Tris/EDTA.

10.2 DNA Isolation:

You can use your method of choice for DNA isolation.

10.3 DNA Storage:

Isolated genomic DNA can be stored at 4°C or -20°C until used.

11. ASSAY PROCEDURE

11.1 Bisulfite Conversion

11.1.1 For each 0.2 mL PCR tube, add 110 µL of the mixed Conversion/Denaturation solution followed by adding 1-5 µL of DNA solution.

Note: *If DNA volume is large and concentration is lower than 10 ng/µL, it is recommended to concentrate DNA prior to bisulfite treatment.*

11.1.2 Tightly close the PCR tubes and place them in a thermal cycler with heated lid. Program and run the thermal cycler at 95°C for 20 min. Meanwhile, insert the number of F-Spin Columns ("column") into F-Collection Tubes ("collection tube") as needed by your experiment.

11.2 Converted DNA Clean-Up

11.2.1 Add 250 µL of Capture Solution to each column. Then transfer the samples from each PCR tube (from Step 11.1.2) to each column containing the Cature Solution. Centrifuge at 12,000 rpm for 30 sec. Remove columns from collection tubes and discard the flowthrough. Place columns back into collection tubes.

11.2.2 Add 200 µL of 90% ethanol solution to each column. Centrifuge at 12,000 rpm for 20 sec.

11.2.3 Add 60 µL of the Final Desulphonation Buffer to each column. Allow columns to sit for 8 min at room temperature, then centrifuge at 12,000 rpm for 20 sec. Remove columns from collection tubes and discard the flowthrough. Place columns back into collection tubes.

11.2.4 Add 200 µL of 90% Ethanol to each column. Centrifuge at 12,000 rpm for 20 sec. Remove columns from collection tubes and discard the flowthrough. Place columns back into collection tubes. Add 200 µL of 90% Ethanol to each column again and centrifuge at 12,000 rpm for 30 sec.

11.2.5 Insert each column into a new 1.5 mL tube. Add 10-20 µL of Elution Solution directly to each column's filter membrane. Centrifuge at 12,000 rpm for 30 sec to elute converted DNA.

11.2.6 Modified DNA is now ready for use, or storage at or below -20°C for up to 6 months. We recommend using 1-2 µL of the DNA for each real time qPCR and 2-4 µL for each end-point PCR.

12. ANALYSIS

Methylation specific-real time PCR can be performed using your own successful method to analyse the DNA produced.

13. TROUBLESHOOTING

Problem	Cause	Solution
DNA is Poorly Modified	Poor DNA quality (DNA is severely degraded)	Check if the sample DNA 260/280 ratio is between 1.6-1.9 and if DNA is degraded by running gel
	Too little DNA or too much DNA (i.e., < 100 pg or >1 µg)	Increase or decrease input DNA to within the correct range, or to the optimal amount of 50-200 ng
	Template contains high GC region or secondary structure	Increase the thermal cycler program time by 5-10 min in Step 11.1.2
	Thermal cycling condition is incorrect	Check for appropriate temperature or thermal cycling conditions
	Insufficient DNA cleaning.	Ensure that 12 µL of Desulphonation Solution is added into every 1 mL of 90% Ethanol in Step 9.3
	Conversion Mix Solution contains precipitates	Check if there are precipitates in the bottle of Conversion Mix Solution prior to adding it to the tube. If so, shake the bottle until re-dissolved

Problem	Cause	Solution
DNA is Poorly Modified	Conversion Mix Solution was contaminated by other chemicals or affected by long-term exposure to air	Check if Conversion Mix Solution has any color change (deep yellow or brown) or indissoluble precipitates. If so, use/order new Conversion Mix Solution solution.
	Kit is not stored or handled properly	Store all components of the kit at room temperature. Tightly cap the Conversion Mix Solution after each opening or use
Elution Contains Little or No DNA	Poor input DNA quality (degraded)	Check if DNA is degraded by running gel
	Capture Solution is not added into the sample.	Ensure that Capture Solution is added in Step 11.2.1
	Concentration of ethanol solution used for DNA clean-up is not correct	Use 90% Ethanol for DNA clean-up
	Sample is not completely passed through the filter	Centrifuge for 1 min at 12,000 rpm or until the entire sample has passed through the filter membrane
Poor Results in Downstream Methylation-Specific PCR	Little or no PCR product even in positive control	Ensure that all PCR components were added and that suitable PCR program is used (PCR cycle should be >40)

Problem	Cause	Solution
Poor Results in Downstream Methylation-Specific PCR	Little or no PCR product even in positive control	PCR primers and probes were not appropriate or were incorrectly designed. Ensure the primer and probes are suitable for MS-PCR and the target regions to be amplified are less than 250 bps
		Ensure the amount of template DNA used in PCR was sufficient
	Significant non-specific PCR products	Failed bisulfite conversion. Ensure that all steps of the modification and cleanup protocol were followed and that input DNA amount is within the recommended range
		Primers and probes are not specific for converted DNA and target genes. Check the primer and probe design

14. NOTES

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500
Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp