ab117126 – Bisulfite Conversion Kit – Whole Cell

Instructions for Use

For bisulfite conversion directly on a cell or tissue sample.

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

Version 1 Last Updated 1 February 2019

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1. BACKGROUND

DNA methylation involves the course in which DNA methyltransferases (DNMTs) transfer a methyl group from S-adenosyl-L-methionine to the fifth carbon position of the cytosines. Aberrant DNA methylation is mainly found in 5'-CpG-3' dinucleotides within promoters or in the first exon of genes, which is an important path-way for the repression of gene transcription in diseased cells. It is well demonstrated that DNA methylation plays an important role in the regulation of gene expression, tumorigenesis, and other genetic and epigenetic diseases such as cancer.

There have been many methods for the detection of DNA methylation. Most of them require a bisulfite-based DNA modification before starting methylation assays such as MSP, sequencing, restriction analysis, and others. The bisulfite-based DNA modification is used to discriminate between cytosine and methylated cytosine, in which bisulfite salt converts cytosine residues to uracil in single-stranded DNA, while methylated cytosine remains the same. All current methods for DNA modification need to use isolated DNA as starting material, which leads to the inability to achieve enough modified DNA in tiny amounts of tissue or cell samples. It is common that only minute amounts of tissue or cell samples can be available in biomedical research, high throughput biomarker/drug screening, and pathological diagnosis. These kinds of samples may include tissue biopsy, microdissection samples, cells contained in body fluids, cells cultured in 96 and 384 well plates, and early embryonic cells/oocytes. Thus, direct DNA modification from whole cells or tissues would give an advantage to efficiently utilize these kinds of samples. It could also generate a greater yield of modified DNA because of avoiding DNA loss caused by DNA isolation/purification prior to bisulfite modification.

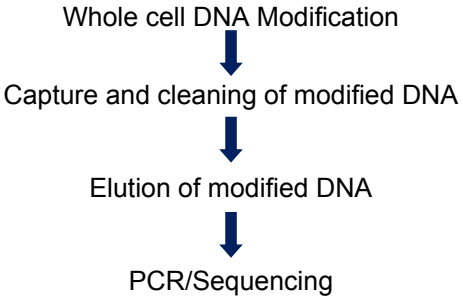
To address this problem ab117126 provides a useful tool to modify DNA directly from the cells or tissues.

The kit has the following advantages and features:

- Fast results: streamlined 3 hour procedure from cells/tissues to modified DNA.
- Completely converts unmethylated cytosine into uracil: modified DNA > 99.9%
- The lowest degradation of DNA in the modification process: more than 90% of DNA loss can be prevented with unique DNA protecting buffer.

The Bisulfite Conversion Kit - Whole Cell contains all reagents required for bisulfite conversion directly on a cell or tissue sample. The kit allows DNA to be isolated from cells or tissues, denatured and bisulfite modified simultaneously in the same tube with the specific reaction buffer under the thermodynamic condition. In the modification process, bisulfite reagent reacts specifically with single-stranded DNA, thereby deaminating cytosine and creating a uracil residue. The unique DNA protection reagents contained in the modification buffer can prevent the chemical and thermophilic degradation of DNA in the bisulfite treatment. The non-toxic modified DNA capture buffer enables DNA to tightly bind to the column filter, thus DNA cleaning can be carried out on the column to effectively remove residual sodium bisulfite and salts. Modified DNA can then be eluted and stably stored at –20°C for up to 2 months.

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.

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.

5. MATERIALS SUPPLIED

Item	40 Reactions	80 Reactions	Storage Condition (Before Preparation)
Digestion Powder	1 vial	1 vial	-20°C
Digestion Solution	0.1 mL	0.2 mL	RT
Cell Collection Buffer	1 mL	2 mL	RT
DNA Modification Powder	4 vials	8 vials	RT
DNA Modification Buffer	5 mL	10 mL	RT
Balance Buffer	0.4 mL	0.8 mL	RT
DNA Binding Buffer	14 mL	28 mL	RT
Modified DNA Elution	1 mL	2 mL	RT
F-Spin Column	40	80	RT
F-Collection Tube	40	80	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
- 15 mL Conical Tubes
- 100% Ethanol
- 70% Ethanol
- 90% Ethanol
- Isopropanol
- Distilled water
- Desktop centrifuge (up to 14,000 rpm)
- Trypsin/EDTA Solution(TE)

**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 **Final Digestion Solution**

Add 50 µL of Digestion Solution to Digestion Powder vial. Stored at 4°C as soon as it is dissolved for up to 6 months.

9.2 **70% Ethanol**

Add 3 mL of distilled water to 7 mL of Ethanol.

9.3 **90% Ethanol**

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

9.4 **DNA Cleaning Solution**

Add 10 µL of Balance Buffer to 1 mL of 90% Ethanol.

9.5 **DNA Modification Solution**

Add 1 mL of DNA Modification Buffer to 1 vial of DNA Modification Powder, followed by adding 60 µL of Balance Buffer. The prepared solution should be used immediately, unless it is stored at –20°C away from light (up to one week). Frozen solution must be thawed at room temperature and vortexed for 2 minutes prior to use.

10. SAMPLE PREPARATION

10.1 **For adherent cultures**

Cells are detached by trypsinization and collected by centrifugation. Add 10 µL of Cell Collection Buffer to re-suspend the cells and transfer into a 0.2 mL PCR tube.

10.2 **For body fluids(cerebro-spinal fluid, ascite, saliva, and urine)**

Simply collect cells by centrifugation. Add 10 µL of Cell Collection Buffer to re-suspend the cells and transfer into a 0.2 mL PCR tube.

10.3 **For tissue biopsy**

Add 10 µL of Cell Collection Buffer into a 0.2 ml PCR tube and then add the sample to the PCR tube containing Cell Collection Buffer.

10.4 **For early embryonic cells or oocytes**

Add 10 µL of Cell Collection Buffer into a 0.2 mL PCR tube and then directly collect the cells into the PCR tube containing Cell Collection Buffer.

10.5 **For tissues from fresh sections**

add 10 µL of Cell Collection Buffer into a 0.2 mL PCR tube. Remove the tissue area you need from slide (0.2-2 mm^2 which represent about 200-2000 cells, assuming the section is 5 µm thick) and add it into the PCR tube containing Cell Collection Buffer.

10.6 **For tissues from formalin-fixed, paraffin-embedded tissue sections**

Remove the paraffin first with deparaffin reagents according to your own successful protocols, or according to the following procedures:

Drop the slide into 100% xylene at room temperature for 5 minutes. Repeat once with new xylene.

Drop the slide into 100% Ethanol, 95% Ethanol, and 70% Ethanol for 5 minutes each. Air dry the slide. Cut the tissue area you need from the slide (0.2-2 mm^2) and add it into the PCR tube containing 10 µL of Cell Collection Buffer.

10.7 **For microdissection samples from fresh or frozen tissue sections**

Add 3-5 µL of Cell Collection Buffer into the center of the cap of a 0.5 mL PCR tube. Place the cap on the tissue to be microdissected and capture cells (100-1000 cells) into the cap. Remove the cap and place it back to the PCR tube containing 10 µL of Cell Collection Buffer.

Note: *If cap is dried, add an additional 3-5 µL of Cell Collection Buffer into the cap before placing it back to the PCR tube.*

Centrifuge at 12,000 rpm for 30 seconds to move the cells down to the solution in the tube. Transfer the solution containing cells into a new 0.2 mL PCR tube.

11. ASSAY PROCEDURE

11.1 Add 1 µL of the Final Digestion Solution to the PCR tubes containing the samples and place the tubes in a thermal cycler with the program of 65°C for 45 minutes. Vortex until solution is clear or saturated (about 2 minutes).

11.2 Add 110 µL of the mixed DNA Modification Solution to each PCR tube containing the sample. Place the tube in a thermal cycler (with heated lid) and program the thermal cycler as follows:

99°C for 20 minutes
65°C for 90 minutes
99°C for 10 minutes

11.3 Modified sample can then be held at 25°C in the thermal cycler up to 4 hours without loss of performance.

11.4 Place a spin column into a 2 mL collection tube. Add 200 µL of DNA Binding Solution to the column. Transfer the sample (from step 11.3) to the column containing DNA Binding Solution, followed by adding 100 µL of 100% Isopropanol to the column. Sit for 2 minutes at room temperature and centrifuge at 12,000 rpm for 20 seconds. Remove the column from the collection tube and discard the flowthrough. Replace column to the collection tube.

11.5 Add 200 µL of 70% Ethanol to the column, and centrifuge at 12,000 rpm for 25 seconds.

11.6 Add 50 µL of the DNA cleaning solution to the column. Sit for 10 minutes at room temperature, then centrifuge at 12,000 rpm for 20 seconds.

11.7 Add 200 µL of 90% Ethanol to the column, centrifuge at 12,000 rpm for 20 seconds. Remove the column from the collection tube and discard the flowthrough. Replace column to the collection tube. Add 200 µL of 90% Ethanol to the column again and centrifuge at 12,000 rpm for 40 seconds.

11.8 Place the column in a new 1.5 mL vial. Add 8-18 µL of Modified DNA Elution, depending on the amount of starting materials, directly to the column filter. Centrifuge at 12,000 rpm for 20 seconds to elute modified DNA.

11.9 Modified DNA is now ready for methylation amplification or storage at -20°C for up to 2 months

12. ANALYSIS

Typical Results

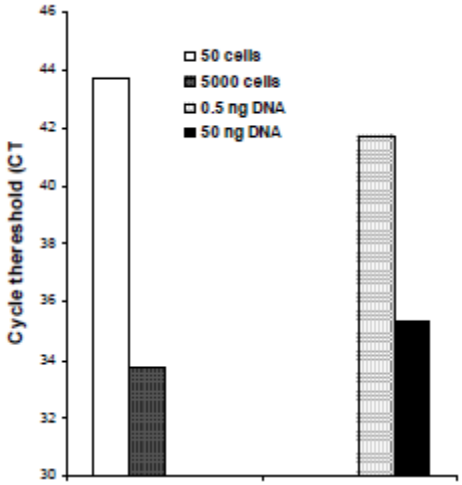


Figure 1. MCF7 cells were modified using ab117126. 10 µl of modified DNA were eluted and 2 µl of elution were used in real time PCR. A pair of primers and a probe designed to amplify both methylated and unmethylated alleles of β-actin was used.

13. TROUBLESHOOTING

Problem	Cause	Solution
DNA is Poorly Modified	Insufficient cell/tissue lysis	Increase incubation time to 60-90 minutes at 65°C at step 11.2
	Template contains high GC region or secondary structure	Increase bisulfite reaction time (65°C) to 120 minutes at step 11.2
	Thermal cycling condition is incorrect	Check if the thermal cycling condition is set according to the protocol
	Bisulfite reaction components are not mixed correctly	Ensure that each component is added correctly
	Insufficient DNA cleaning	Ensure that sufficient Balance Buffer is added into 90% Ethanol
	Incorrect storage of Final DNA Modification Solution	Ensure that Final DNA Modification Solution is stored at –20°C for no more than 2 weeks
Elution Contains Little or No DNA	Poor starting material quality (Ex: FFPE sample contains fragmented DNA)	Check if starting material is in good quality
	Too little starting material (ex: < 50 cells)	Increase starting material

Elution Contains Little or No DNA	DNA Binding Buffer is not added into the sample.	Ensure that DNA Binding Buffer is added as described in step 11.4
	DNA cleaning solution is prepared incorrectly at step 9.4 of the protocol	Ensure that Balance Buffer is added into 90% Ethanol.
	The column is not washed with 90% Ethanol	Ensure that wash solution is 90% Ethanol.
	Sample is not completely passed through the filter	Increase centrifuge time to 1 minute at steps 11.4- 11.8
Elution Contains Both Unmodified and Modified DNA	Amount of cells/tissue used is out of recommended range	Adjust the amount of starting cells/tissue to recommended range
	Template with high G-C content	Increase bisulfite reaction time (65°C) to 120 minutes at step 11.2
Poor Methylation Specific-PCR Products	PCR components are not sufficiently added	Check if all PCR components were added

14. NOTES

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