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PRODUCT INFORMATION

Thermo Scientific
EpiJET Bisulfite Conversion Kit
#K1461

www.thermoscientific.com/onebio

#___
Lot __
Expiry Date __

CERTIFICATE OF ANALYSIS

The kit is functionally tested by analyzing bisulfite converted fully methylated (by M.SssI) Human Genomic DNA . Performance of the kit is assayed by qPCR with primers, specific for converted and unconverted DNA. The kit passes QC requirements for DNA recovery and conversion efficiency.

Quality authorized by:  Jurgita Zilinskiene

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KIT COMPONENTS

Component	#K1461 50 rxns
Modification Reagent	5 vials
Modification Solution I	1.2 mL
Modification Solution II	0.35 mL
Binding Buffer for Bisulfite Conversion Kit	25 mL
Wash buffer (conc.) for Bisulfite Conversion Kit	9 mL
Desulfonation Buffer (conc.)	3.5 mL
Elution Buffer	1.5 mL
DNA Purification Micro Columns & Collection Tubes	50

STORAGE

All components of the kit should be stored tightly capped at room temperature (15-25 ºC). Modification Reagent is light sensitive, therefore minimize its exposure to light. Prepared Modification Reagent solution can be stored for up to one week at 4 ºC or 4 weeks in the dark at -20 ºC.

DESCRIPTION

Thermo Scientific™ EpiJET™ Bisulfite Conversion Kit is designed for simple and reliable bisulfite conversion of DNA for methylation analysis. In the bisulfite reaction all unmethylated cytosines are deaminated and converted to uracils, while methylated cytosines remain unchanged. Bisulfite converted unmethylated cytosines are detected as thymines in the following PCR:

Template	N-^{M}C-G-N-C-G-N
After bisulfite conversion	N-^{M}C-G-N-U-G-N
After PCR	N- C-G-N-T-G-N

The EpiJET Bisulfite Conversion Kit integrates the thermal DNA denaturation and the bisulfite conversion reaction into one step. This step can be performed following the long (Protocol A, 160 min) or short protocol (Protocol B, 30 min). The converted DNA is then bound to a membrane of a micro column for on-column desulfonation and subsequent DNA purification steps. The converted DNA is eluted in a low volume of Elution Buffer and is suitable for a number of techniques used for the methylation status analysis, including PCR, qPCR, COBRA, and sequencing.

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- Ethanol 96-100%, molecular biology grade
- Water, molecular biology grade
- Pipettes and pipette tips
- Thin-walled PCR tubes
- Thermal cycler with heated lid
- Sterile 1.5 mL microcentrifuge tubes for the elution step
- Microcentrifuge
- Disposable gloves

IMPORTANT NOTES

- DNA purity is an important factor for successful bisulfite conversion. We recommend using kits such as Thermo Scientific™ GeneJET™ Genomic DNA Purification Kit (cat. #K0721/2) for genomic DNA purification.
- Starting DNA amounts from 50 pg up to 2 µg can be used for DNA conversion using the kit. Nevertheless, for optimal results, use 200 ng - 500 ng of input DNA. High input DNA amounts may result in incomplete bisulfite conversion for some GC-rich regions.
- **Do not use** Phusion, Pfu or other proofreading DNA polymerases, which are inhibited by uracil.
- We recommend using hot start Taq DNA polymerases such as Thermo Scientific™ Maxima™ Hot Start Taq DNA Polymerase (cat. #EP0601/2/3) for converted DNA amplification.
- Since the bisulfite converted DNA strands are no longer complementary, this DNA stays mainly in a single stranded form and resembles RNA. For converted DNA concentration measurements use a value of 40 µg/mL for absorbance at 260 nm = 1.0.
- Two alternative protocols are provided for DNA denaturation/bisulfite conversion reaction step:
  - Protocol A results in DNA conversion efficiency > 99%, is more sensitive and gives lower DNA degradation levels than Protocol B. This protocol is recommended for all routine epigenetic applications.
  - Protocol B results in DNA conversion efficiency > 95% and is much faster than Protocol A (30 min vs. 160 min for DNA denaturation/bisulfite conversion step). Modified DNA is suitable for PCR of amplicons up to 300 bp. This protocol is not recommended for small DNA amounts (less than 50 ng of input DNA). It is the best for fast DNA methylation screening.

REAGENT PREPARATION

Wear disposable gloves during reagent preparation and DNA conversion procedure.

- **Preparation of Modification Reagent.**

Modification Reagent is supplied as a dry mixture and needs to be dissolved before use. The amount of Modification Reagent in each vial provided is sufficient for 10 DNA conversion reactions. Prepare only as many tubes as needed for current experiment. For 10 conversion reactions:

1. Add 0.9 mL of molecular biology grade water, 200 µL of Modification Solution I and 60 µL of Modification Solution II to one vial of dry Modification Reagent.
2. Dissolve Modification Reagent by inverting for about 10 min (trace amounts of undissolved bisulfite may remain).

For best results, the prepared reagent should be used immediately.
Modification Reagent solution can be stored for up to one week at 4ºC or 4 weeks in the dark at -20ºC. Modification Reagent solution taken from the refrigerator must be warmed to room temperature and vortexed prior to use.

- **Preparation of Wash Buffer:** add 25 mL of 96-100% ethanol to the 9 mL of Wash Buffer (conc.) before use.
- **Preparation of Desulfonation Buffer:** add 10 mL of 96-100% ethanol to the 3.5 mL of Desulfonation Buffer (conc.) before use.

After the ethanol has been added, mark the check box on the bottle's cap to indicate the completed step.

PROTOCOL

1. Add 20 µL of DNA sample containing 200-500 ng of purified genomic DNA into a PCR tube. If a volume of DNA sample is less than 20 µL, add water, molecular biology grade, to DNA sample up to 20 µL.
2. Add 120 µL of prepared **Modification Reagent** solution to 20 µL of DNA sample in a PCR tube. Mix the sample by pipetting up and down, then centrifuge the liquid to the bottom of the tube.
3. Place PCR tubes into a thermal cycler and proceed with Protocol A or B to perform denaturation and bisulfate conversion of DNA:

   Protocol A*
   1) 98°C/ 10 min.
   2) 60°C/ 150 min.
   3) Proceed immediately to step 4. Alternatively, converted DNA can be stored at 4 °C overnight.

   Protocol B**
   1) 98°C/ 30 min.
   2) Proceed immediately to step 4. Alternatively, converted DNA can be stored at 4 °C overnight.

   *Protocol A results in DNA conversion efficiency > 99%, is more sensitive and gives lower DNA degradation levels than Protocol B. This protocol is recommended for all routine epigenetic applications.

   **Protocol B results in DNA conversion efficiency > 95% and is much faster than Protocol A. Modified DNA is suitable for PCR of amplicons up to 300 bp. This protocol is not recommended for small DNA amounts (less than 50 ng of input DNA), and is best for fast DNA methylation screening.

4. Add 400 µL of **Binding Buffer** to the **DNA Purification Micro Column** placed into the collection tube.
5. Load the converted DNA sample (from step 3) into the **Binding Buffer** in the column, mix completely by pipetting.
6. Centrifuge the micro column placed into the collection tube at 12,000 rpm for 30 seconds. Discard the flow-through. Place the micro column into the same collection tube.
7. Add 200 µL of **Wash Buffer**, prepared with ethanol, to the micro column and centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through. Place the micro column into the same collection tube.
8. Add 200 µL of **Desulfonation Buffer**, prepared with ethanol, to the micro column and let the column stand at room temperature for 20 min.
9. Centrifuge the micro column placed into the collection tube at 12,000 rpm for 30 seconds. Discard the flow-through. Place the micro column into the same collection tube.
10. Add 200 µL of **Wash Buffer**, prepared with ethanol, to the micro column and centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through. Place a micro column into the same collection tube.
11. Add an additional 200 µL of **Wash Buffer**, prepared with ethanol, to the micro column and centrifuge at 12,000 rpm for 60 seconds.
12. Place the column into a clean1.5 ml microcentrifuge tube (not provided). Add 10 µL of **Elution Buffer** to the micro column. Centrifuge at 12,000 rpm for 60 seconds.

***Note**: More than 10 µL (up to 20 µL) of Elution Buffer can also be used for converted DNA elution. If a small amount (< 1 ng) of DNA was used for DNA conversion, elute DNA with 6-8 µL of Elution Buffer. Repeat the elution step twice (for a total volume of 12-16 µL).*

13. Eluted converted DNA is ready for downstream analysis. DNA can be stored at -20 ºC for more than 1 year.

PCR CONDITIONS

- PCR using “hot start” polymerases such as Maxima Hot Start Taq DNA Polymerase (cat. #EP0601/2/3) is strongly recommended for the amplification of bisulfite converted DNA. **Do not use** Phusion, Pfu or other proofreading DNA polymerases, which are inhibited by uracil.
- Use 2-4 µL of eluted DNA for each PCR.
- Optimal amplicon size should be 100-500 bp, but larger amplicons can be also generated after optimization of PCR conditions.
- 35-45 cycles are required for successful PCR amplification of bisulfite converted DNA.
- Use the recommended software, EpiDesigner, http://www.epidesigner.com, or similar to design primers for bisulfite converted DNA PCR.

TROUBLESHOOTING

Problem	Cause and Solution
Little or no DNA in the eluate	**Low purity of genomic DNA used.** • For high quality genomic DNA purification use commercially available genomic DNA purification kits such as GeneJET Genomic DNA Purification Kit (cat. #K0721/2). **Wash Buffer or Desulfonation Buffer was prepared incorrectly.** • Check if buffer concentrates were diluted with the correct volumes of ethanol (96-100%). **Improper DNA amount used for DNA conversion reaction.** • Check if amount of DNA used is outside of the recommended range.
Low conversion efficiency	**Incorrect bisulfite conversion reaction conditions.** • Check if thermal cycling conditions of Protocol A or B were correct. **Low purity of genomic DNA used.** • Ensure the sample DNA 260/280 ratio is between 1.6-1.9. For high quality genomic DNA purification use commercially available genomic DNA purification kits such as GeneJET Genomic DNA Purification Kit (cat. #K0721/2). **Improper storage conditions.** • Check if reagents of the kit were stored at room temperature. Also, ensure that Modification Reagent was stored at 4°C or at -20°C after resuspension.
No product or poor results in downstream PCR	**Improper reaction setup.** • Make sure that all PCR components were added to the tube and cycling conditions were correct. • PCR cycle number should be > 35. • Verify if the initial denaturation/activation step was applied for hot start PCR. **Incorrect polymerase was used.** • Use Taq polymerases, such as Maxima Hot Start Taq DNA Polymerase (cat. #EP0601/2/3), for bisulfite converted DNA amplification. Phusion, Pfu or other proofreading DNA polymerases, which are sensitive to uracils, are not suitable for amplification of bisulfite-treated DNA. **Improper PCR primers.** • PCR primers were not designed correctly. Use designated software, such as EpiDesigner, http://www.epidesigner.com, or similar, to design primers for bisulfite converted DNA amplification. **Sample DNA degradation.** • Ensure that sample DNA is handled and stored correctly. **Eluted DNA is contaminated with Wash Buffer.** • Before adding of the Elution Buffer to the micro column (step 12) ensure that there are no droplets of Wash Buffer left on the micro column (this could happen due to incautious handling of the micro column and collection tube containing Wash Buffer after centrifugation; step 11). If this happens place a column into a new 1.5 mL microcentrifuge tube (not provided) and centrifuge at 12,000 rpm for 60 seconds to ensure that all residual Wash Buffer was eluted from the micro column. After centrifugation proceed with step 12.

Modification Reagent

Danger

Hazard-determining components of labelling**: sodium metabisulfite**

Hazard statements

H302+EUH031 Harmful if swallowed. Contact with acids liberates toxic gas.
H318 Causes serious eye damage.

Precautionary statements

P280 Wear protective gloves/protective clothing/eye protection/face protection.
P264 Wash thoroughly after handling.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a POISON CENTER or doctor/physician.
P301+P312 IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

Modification Solution I

Danger

Hazard-determining components of labelling: **sodium hydroxide**

Hazard statements

H314 Causes severe skin burns and eye damage.

Precautionary statements

P260 Do not breathe dust/fume/gas/mist/vapours/spray.
P303+P361+P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a POISON CENTER or doctor/physician.
P405 Store locked up.
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

Modification Solution II

Danger

Hazard-determining components of labelling: **N,N-dimethylformamide**

Hazard statements

H226 Flammable liquid and vapour.
H319 Causes serious eye irritation.
H360D May damage the unborn child.

Precautionary statements

P210 Keep away from heat/sparks/open flames/hot surfaces. - No smoking.
P241 Use explosion-proof electrical/ventilating/lighting/equipment.
P303+P361+P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P405 Store locked up.
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

Binding Buffer for Bisulfite Conversion Kit

Xn Harmful

Hazard-determining components of labelling: **guanidinium chloride**

Risk phrases

R20/21/22 Harmful by inhalation, in contact with skin and if swallowed.
R32-52/53: Contact with acids liberates very toxic gas. Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

Safety phrases

23 Do not breathe gas/fumes/vapour/spray.
26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
36/37 Wear suitable protective clothing and gloves.
60 This material and its container must be disposed of as hazardous waste.

Desulfonation Buffer

Danger

Hazard-determining components of labelling: **sodium hydroxide**

Hazard statements

H314 Causes severe skin burns and eye damage.

Precautionary statements

P260 Do not breathe dust/fume/gas/mist/vapours/spray.
P303+P361+P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing.
Rinse skin with water/shower.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a POISON CENTER or doctor/physician.
P405 Store locked up.
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.