Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific EpiJET DNA Methylation Analysis Kit (TaqI/HpyF30I)

#K1451 **200 rxns**

Lot ___ **Expiry Date _**

5'...**T C G A**...3'
3'...**A G C T**...5'

Store at -20°C

www.thermoscientific.com/onebio

Kit components

Component	#K1451 200 rxns
Epi HpyF30I	200 µL
Epi TaqI	200 µL
10X EpiBuffer 2	1.2 mL
Control DNA Unmethylated (pUC19/SmaI) (0.5 µg/µL)	20 µL
Control DNA CpG Methylated (mpUC19/SmaI) (0.5 µg/µL)	20 µL

CERTIFICATE OF ANALYSIS

The kit is tested in Epi HpyF30I and Epi TaqI digestion of methylated and unmethylated pUC19/SmaI DNA mixed with human blood genomic DNA. Unmethylated plasmid DNA is completely cleaved in one hour with both enzymes in presence of genomic DNA. Methylated plasmid DNA is not cleaved by Epi HpyF30I, but cleaved with Epi TaqI in presence of genomic DNA.

Quality authorized by: Jurgita Zilinskiene

Description

5-methylcytosine is a prominent epigenetic DNA modification which plays an important role in up- and down-regulation of gene expression. In mammalian cells 5-methylcytosines are mainly found in 5'-CpG-3' dinucleotides and are enriched in CpG islands, the GC-rich regions most often located in gene promoters. MspI/HpaII enzyme pair with 5'-CCGG-3' recognition sequence has multiple targets in such regions and is routinely used for cytosine methylation analysis. Outside the CpG islands the CCGG motives are much less abundant and limit the usability of the aforementioned enzyme pair.

The Thermo Scientific™ EpiJET™ DNA Methylation Analysis Kit (TaqI/HpyF30I) uses another pair of restriction enzymes to analyze the DNA methylation status in gene bodies and other low-GC content genomic regions. Epi HpyF30I and Epi TaqI are isoschizomers with TCGA specificity. Methylation of cytosine in the 5'-TCGA-3' tetranucleotide blocks the cleavage with Epi HpyF30I but does not affect the cleavage with Epi TaqI.

The Epi HpyF30I and Epi TaqI enzymes are specially formulated for the epigenetic studies to complete genomic DNA digestion in 1 hour.

Application

CpG methylation analysis at the 5'-TCGA-3' loci.

Important Notes

- To minimize the possibility of pipetting errors during the DNA digestion, prepare enough reaction master mix (including DNA sample, but not including Epi HpyF30I and Epi TaqI) for three reactions plus 10% extra. Mix thoroughly and dispense the mixture into three sterile tubes (19 µL each). Add 1 µL of nuclease-free water into tube **1**, add 1 µL of Epi HpyF30I into tube **2**, add 1 µL of Epi TaqI into tube **3** and follow protocol recommendations.
- For control reactions prepare two distinct reaction master mixes (one with Control DNA Unmethylated (pUC19/SmaI) and the other with Control DNA CpG Methylated (mpUC19/SmaI)). Mix thoroughly and dispense each mixture into three sterile tubes (19 µL each). Add 1 µL of nuclease-free water into tubes **1** and **4**, add 1 µL of Epi HpyF30I into tubes **2** and **5**, add 1 µL of Epi TaqI into tubes **3** and **6** and follow protocol recommendations.
- DNA sample quality is important for efficient digestion by restriction endonucleases. We recommend use of spin column based kits such as the Thermo Scientific GeneJET™ Genomic DNA Purification Kit (#K0721/2) for genomic DNA purification.
- Reactions can be scaled up, incrementally increasing reaction volume by 10 µL and enzyme amount by 1 µL for every microgram of DNA used. For example, to digest 2 µg of genomic DNA use 2 µL of Epi HpyF30I or Epi TaqI in 30 µL reaction volume. If digesting less than 1 µg, or if DNA concentration is high enough (≥125 ng/µL) to accommodate 1 µg into total volume of 8 µL, reaction volume can be scaled down to 10 µL.

Protocol

- Read the Important Notes section before starting.

1. Mix the following components in sterile microcentrifuge tubes:

Component	Undigested DNA	Digested with Epi HpyF30I	Digested with Epi TaqI
	1	2	3
Sample DNA	up to 1 µg*	up to 1 µg*	up to 1 µg*
10X Epi Buffer 2	2 µL	2 µL	2 µL
Epi HpyF30I	-	1 µL	-
Epi TaqI	-	-	1 µL
Water, nuclease-free (#R0581)	to 20 µL	to 20 µL	to 20 µL
Total volume	20 µL	20 µL	20 µL

2. Incubate all samples at 60°C for 1 hour.
3. Terminate the reactions by incubation at 90°C for 10 min.

- To prepare protein free DNA, extract with phenol/chloroform.

*For methylation analysis by qPCR use 0.04-0.4 µg of sample DNA.

Control Reaction

- To assess DNA sample quality and potential inhibitory effects, perform control reaction using provided unmethylated and methylated control DNA.
- Read the Important Notes section before starting.

1. Mix the following components in sterile microcentrifuge tubes:

Component	Undigested DNA	Digested with Epi HpyF30I	Digested with Epi TaqI
	1	2	3
Sample DNA	0.5 µg	0.5 µg	0.5 µg
Control DNA Unmethylated (pUC19/SmaI)	0.5 µg	0.5 µg	0.5 µg
10X Epi Buffer 2	2 µL	2 µL	2 µL
Epi HpyF30I	-	1 µL	-
Epi TaqI	-	-	1 µL
Water, nuclease-free (#R0581)	to 20 µL	to 20 µL	to 20 µL
Total volume	20 µL	20 µL	20 µL

Component	Undigested DNA	Digested with Epi HpyF30I	Digested with Epi TaqI
	4	5	6
Sample DNA	0.5 µg	0.5 µg	0.5 µg
Control DNA CpG Methylated (mpUC19/SmaI)	0.5 µg	0.5 µg	0.5 µg
10X Epi Buffer 2	2 µL	2 µL	2 µL
Epi HpyF30I	-	1 µL	-
Epi TaqI	-	-	1 µL
Water, nuclease-free (#R0581)	to 20 µL	to 20 µL	to 20 µL
Total volume	20 µL	20 µL	20 µL

2. Incubate all samples at 60°C for 1 hour.
3. Terminate the reactions by incubation at 90°C for 10 min.
4. Analyze the reaction products by DNA electrophoresis on an agarose gel.

Typical results are presented in Fig 1.

Jurkat genomic DNA (uppermost band) is only partially digested by Epi HpyF30I and fully fragmented by Epi TaqI that is not sensitive to CpG methylation. Unmethylated plasmid DNA is digested by both enzymes, while methylated plasmid DNA is susceptible only to Epi TaqI digestion. Any inhibition of control DNA cleavage by restriction endonucleases indicates the presence of inhibitors. In such a case repurification of sample DNA is recommended

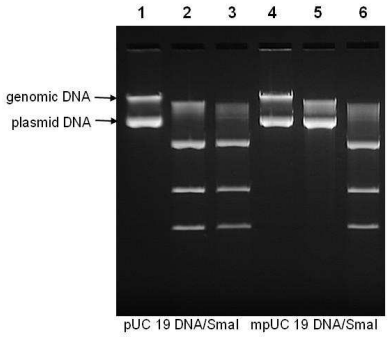


Figure 1. Digestion of genomic and control DNA by Epi TaqI and Epi HpyF30I
Lanes 1 and 4 – undigested DNA,
Lanes 2 and 5 – DNA digested with Epi HpyF30I,
Lanes 3 and 6 – DNA digested with Epi TaqI,
Lanes 1-3 contain Control DNA Unmethylated (pUC19/SmaI),
Lanes 4-6 contain Control DNA CpG Methylated (mpUC19/SmaI).
Genomic DNA in all lanes is Jurkat gDNA.

Guidelines for qPCR

If CpG methylation at a particular TCGA locus is analyzed by qPCR, follow the recommendations provided below.

- For qPCR set primers that flank the TCGA site of interest.
- Use 1-2 µL (2-40 ng) of template prepared according to the Protocol and follow the manufacturer's recommendations for qPCR reaction set-up and cycling conditions.
- During qPCR setup, it is important to avoid DNA cross-contamination. We recommend using a dedicated set of pipettes for qPCR to minimize contamination.
- The accuracy of qPCR is highly dependent on accurate pipetting and thorough mixing of individual reaction components. Take extra care to avoid pipetting errors during qPCR set up and when preparing templates for qPCR. Use of 2-3 technical replicates is highly recommended.
- For calculation of 5-mC percentage use the formula provided in the Calculation of 5-hmC Percentage section. To determine the PCR efficiency value prepare a standard calibration curve by diluting the "Undigested DNA" sample.

Recommendations for qPCR

- The parameters below are recommended for qPCR using Thermo Scientific Maxima™ SYBR Green/ROX qPCR Master Mix (#K0221/2/3).
- To minimize the possibility of pipetting errors, prepare a reaction master mix by adding the following components (excluding template DNA) for each 20 µL reaction to a tube at room temperature:

Maxima SYBR Green/ROX qPCR Master Mix (2X)	10 µL
Forward Primer	0.3 µM
Reverse Primer	0.3 µM
Sample DNA prepared according to the Protocol	1-2 µL (2-40 ng)
Water, nuclease-free	to 20 µL
Total volume	20 µL

- Mix the reaction master mix thoroughly and dispense the desired volume into PCR tubes or plates.
- Add template DNA (1-2 µL (2-40 ng)) prepared according to the Protocol to the individual PCR tubes or wells containing master mix. Centrifuge briefly if needed.

Note. *To minimize inaccuracies associated with pipetting smaller volumes we recommend to dilute template DNA 3-fold and use 3-6 µL.*

- Program the thermal cycler according to the recommendations below:

Step	Temperature, °C	Time	Number of Cycles
Initial denaturation	95	10 min	1
Denaturation	95	15 sec	40
Annealing	60*	30 sec	
Extension	72	30 sec	

* use the optimal annealing temperature for your primer pair.

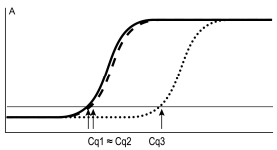
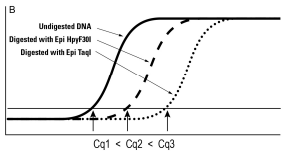
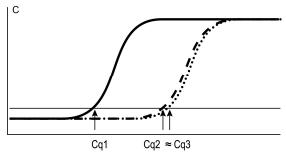
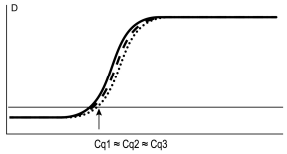
- Use two-step cycling protocol, if the optimal annealing temperature for your primer pair is 60°C:

Step	Temperature, °C	Time	Number of Cycles
Initial denaturation	95	10 min	1
Denaturation	95	15 sec	40
Annealing/ Extension	60	60 sec	

Note. *Melting curve analysis may be performed to verify the specificity of the PCR product. Primer-dimers may occur during PCR if the primer design is not optimal. The dimers are distinguished from the specific product through their lower melting point.*

Interpretation of qPCR Results

For interpretation of qPCR results see the picture provided below.

	If Cq3 – Cq1 ≥ 4.5* and Cq1 ≈ Cq2** **Full methylation**. Methylation blocked the Epi HpyF30I cleavage. TCGA site is fully methylated.
	If Cq3 – Cq1 ≥ 4.5* and Cq1 < Cq2 < Cq3 **Partial methylation**. Epi HpyF30I cleavage level represents methylation level in the TCGA site. Calculates into 5- mC percentage
	If Cq3 – Cq1 ≥ 4.5* and Cq2 ≈ Cq3** **No methylation**. TCGA site does not contain 5-mC bases. Methylation does not affect the cleavage.
	Cq1≈ Cq2 ≈ Cq3** **No cleavage.** No Epi HpyF30I/Epi TaqI recognition site.

Cq1 - threshold cycle of "Undigested DNA" sample (continuous curve)
Cq2 - threshold cycle of "Digested with Epi HpyF30I" DNA sample (dashed curve)
Cq3 - threshold cycle of "Digested with Epi TaqI" DNA sample (dotted curve)

* Cq3 – Cq1 < 4.5 means the incomplete digestion of sample DNA by Epi TaqI. Repurify sample DNA or extend the digestion time.

** ≈ stands for ΔCq ≤ 0.5

Calculation of 5-mC Percentage:

- If Cq3 – Cq1 ≥ 4.5, % of 5-mC is calculated using the formula below:

% of 5-mC =100 / (1 + E)$^{Cq2-Cq1}$

Where:
Cq1 is the threshold cycle of "Undigested DNA" sample
Cq2 is the threshold cycle of "Digested with Epi HpyF30I" sample
Cq3 is the threshold cycle of "Digested with Epi TaqI" sample
E is the PCR efficiency value (%)

- % of 5-mC can not be accurately estimated when:

- Cq3 – Cq1 < 4.5.

This result demonstrates the incomplete digestion of sample DNA by Epi TaqI. Repurify sample DNA or extend the digestion time. For high quality genomic DNA purification use commercially available gDNA purification kits, such as GeneJET Genomic DNA Purification Kit (#K0721/2).

- Cq1 ≈ Cq2 ≈ Cq3.

This result demonstrates the absence of Epi HpyF30I/Epi TaqI recognition sequence.

Patent pending

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.