

Cat. # R100A

For Research Use

TAKARA

EpiScope[®] MSP Kit

Product Manual

v202202Da

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I. Description

EpiScope MSP Kit is a PCR reagent kit designed exclusively for methylation-specific PCR (MSP) analysis in methylation analysis of genomic DNA. A specific enzyme combined with an optimized buffer allows MSP analysis using a bisulfite-treated DNA template containing uracil. This kit provides a greatly improved ability to distinguish methylated/unmethylated template compared with conventional PCR reagents. The reaction system has been optimized for real-time monitoring using TB Green® as an intercalator, and makes it possible to perform both real-time PCR and endpoint PCR reaction, in which amplification is determined by gel electrophoresis, under the same reaction conditions.

II. MSP Principle

The first step is to identify a nucleotide region whose sequence is subject to change by bisulfite treatment depending on the methylation status of the CpG sequence. Next, design two primers for the CpG site of interest, one for methylated CpG DNA (M primer) and the other for unmethylated DNA (UM primer). The last step is PCR amplification.

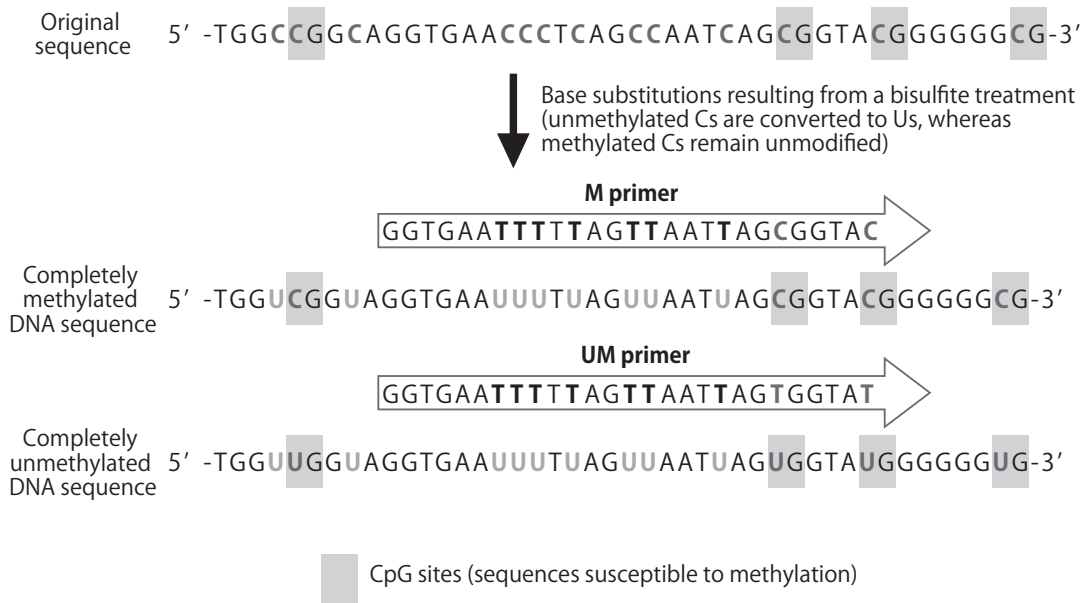


Figure 1. Principle of MSP

III. Components [200 reactions, 50 µl volume per reaction]

(1)	2X MSP Buffer (Mg ²⁺ plus, dNTP plus)*1	1 ml x 5
(2)	MSP Enzyme	240 µl
(3)	TB Green Solution (X100)	100 µl
(4)	ROX Reference Dye (50X conc.)*2	200 µl
(5)	ROX Reference Dye II (50X conc.)*2	200 µl

*1 The Mg²⁺ concentration (2X) is 4 mM, and the dNTP concentration (2X) is 400 µM.

*2 This component is to be used for analyses using a device that corrects fluorescent signals between wells such as the real-time PCR device by Applied Biosystems.

- ◆ Use ROX Reference Dye (50X):
 - StepOnePlus Real-Time PCR System (Thermo Fisher Scientific)
- ◆ Use ROX Reference Dye II (50X):
 - Applied Biosystems 7500 Real-Time PCR System
 - Applied Biosystems 7500 Fast Real-Time PCR System (Thermo Fisher Scientific)
- ◆ Do not use either Reference Dye:
 - Thermal Cycler Dice™ Real Time System III (Cat. #TP950/TP970/TP980/TP990)*3
 - Thermal Cycler Dice Real Time System III/Lite (Cat. #TP900/TP960/TP700/TP760: discontinued)
 - An ordinary PCR device for electrophoretic analysis

*3 Not available in all geographic locations. Check for availability in your area.

IV. Storage

-20°C

Note: It is important to protect TB Green Solution (X100) from light.

V. Materials Required but not Provided**1. Reagents**

- Primers for PCR
- Sterile purified water

2. Materials

- Special reaction tubes or plates
- Micropipettes and tips (autoclave treated)
- Gene amplification system for real time PCR or ordinary PCR device (authorized instruments)

VI. Precautions

- (1) Place all reagents, except TB Green Solution (X100), on ice when preparing the reaction mixture. TB Green Solution (X100) will freeze on ice. Keep it at room temperature protected from light.
- (2) Use fresh disposable tips to avoid any potential contamination between samples when preparing or dispensing reaction mixtures.

VII. Protocol

1. Primer design

We recommend using a primer design tool that is specific for bisulfite-treated sequences. The following design tools are available online, free of charge. (For specific operating instructions, please refer to the help section of each tool.)

The optimum size of amplification products is between 80 and 150 bp (amplifications of up to 200 bp are possible).

MethPrimer

<https://www.urogene.org/methprimer/index1.html>

MethPrimer - Design Primers for Methylation PCRs

Home Protocols Resources FAQ Help

Paste a ORIGINAL source [sequence](#). Try this [Sample sequence](#)
You don't need to modify your sequence (e.g. convert 'C' to 'T') before pasting.

Pick primers for [bisulfite sequencing PCR](#) or [restriction PCR](#).

Pick **MSP** primers.

Use CpG island prediction for primer selection?

Window: 100 Shift: 1 Obs/Exp: 0.6 GC%: 50

Submit Reset

MethPrimer - Design Primers for Methylation PCRs

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General Parameters for Primer Selection

Sequence name (optional):

Target (optional): *start, size", such as (560, 30)

Excluded Regions (optional): *start, size", such as (160, 50)

Number of output pairs (optional): 5

Product Size: Min: 100 Opt: 200 Max: 300

Primer Tm: Min: 50 Opt: 55 Max: 60

Primer Size: Min: 20 Opt: 25 Max: 30

Product CpGs: 4 Primer Poly X: 5

Primer non-CpG 'C's: 4 Primer Poly I: 8

Parameters for MSP primers

3'CpG constraint: 3

CpG in primer: 1

Max Tm difference: 5

Submit Reset

BiSearch

<https://bisearch.enzim.hu/?m=msp>

ACGGACCGACCGCGGTGTGC
TGCCCTGGCGCTGGCGCACAG

Primer Design and Search Tool

Menu

Primer tm

Primer score

Simple search

Primer search, ePCR

Primer design

MSP design

Parameters

Help

Manual

Faq

Manuscripts

ChangeLog

Genome builds

Comments

Statistics

Visitors: 58822

Primer tm: 14545

Primer score: 3603/2142

Search: 3004/1892

Fast PCR: 116236/73226

Primer design: 12488/18852

Search Methylated Specific Primers

Sequence:

Bisulfite: Use sense or antisense chain.

Set search region for: Forward primer: Reverse primer:

Max length of PCR: 400

Min tm diff: 8.0

CpG sites: let only in one or both primers.

Search primers Clear input

Parameters

Primer melting temperature

Primer conc: 1.0 mikromol Glycerol conc: 0.0 %

Potassium conc: 50.0 milimol Ethylen glycol conc: 0.0 %

Magnesium conc: 1.5 milimol Formamid conc: 0.0 %

Primer scoring values

Help

Search the best primer pairs for PCR a sequence

Online help system

The dynamic Online help system informs you about each input field. Move the mouse over the input field you would like to fill in, and read the help here.

2. PCR reaction composition (50 µl reaction)

The reaction composition is the same for real-time PCR and endpoint PCR detection.

Note: Even for endpoint PCR detection, be sure to add TB Green Solution (X100) in the reaction mixture.

Reagent	Amount	Final conc.
2X MSP Buffer	25 µl	1X
PCR forward primer	15 pmol	0.3 µM
PCR reverse primer	15 pmol	0.3 µM
TB Green Solution (X100)	0.5 µl	1X
MSP Enzyme	1.2 µl	
(ROX Reference Dye (50X) or Dye II (50X)*1)	1 µl	1X
DNA template	<5 µl	
Sterile purified water	up to 50 µl*2	

- *1 This component is to be used for analyses using a device that corrects fluorescent signals between wells such as the real-time PCR device by Thermo Fisher Scientific. Please use ROX Reference Dye for StepOnePlus or ROX Reference Dye II for 7500 and 7500 Fast Real-Time PCR System. This component is not required with Thermal Cycler Dice Real Time System series; nor is it required for endpoint PCR detection by electrophoresis.
- *2 Please change the reaction volume appropriately in accordance with the recommended volume for each PCR device.

3. PCR condition

The PCR condition is the same for real-time PCR and endpoint PCR detection.

95°C 30 sec
 ↓
 98°C 5 sec
 55°C 30 sec
 72°C 1 min (up to 200 bp)] 40 - 45 cycles
 ↓
 Melting-curve analysis (for real-time PCR)

Note: The MSP enzyme supplied in this kit is a hot start PCR enzyme that uses an anti-*Taq* antibody that inhibits polymerase activity. Please do not perform the 5 to 15 min activation at 95°C before PCR reaction that is required with other companies' chemically modified hot start PCR enzymes. Unnecessary heat treatment tends to reduce enzyme activity and affect amplification efficiency. Even for the initial denaturation of template, 95°C for 30 sec is generally sufficient.

After reaction completion, perform the required analysis for real-time PCR or an agarose gel electrophoresis for endpoint PCR detection. After electrophoresis, stain the gel in the usual manner with a stain such as ethidium bromide.

VIII. Experimental Example

MSP for the promoter region of each of the *CDH1*, *CDKN2A*, and *MLH1* genes.

Method: With bisulfite-treated methylated HeLa genome DNA and native HeLa genome DNA as the template (30 ng/25 μl reaction for each), MSP was performed for the promoter region of each of the *CDH1*, *CDKN2A*, and *MLH1* genes.

Result: Comparable results were obtained with real-time PCR and endpoint detection. With the native HeLa genome, the CpG regions of *CDH1* are methylated, but the CpG regions of *CDKN2A* and *MLH1* are unmethylated.

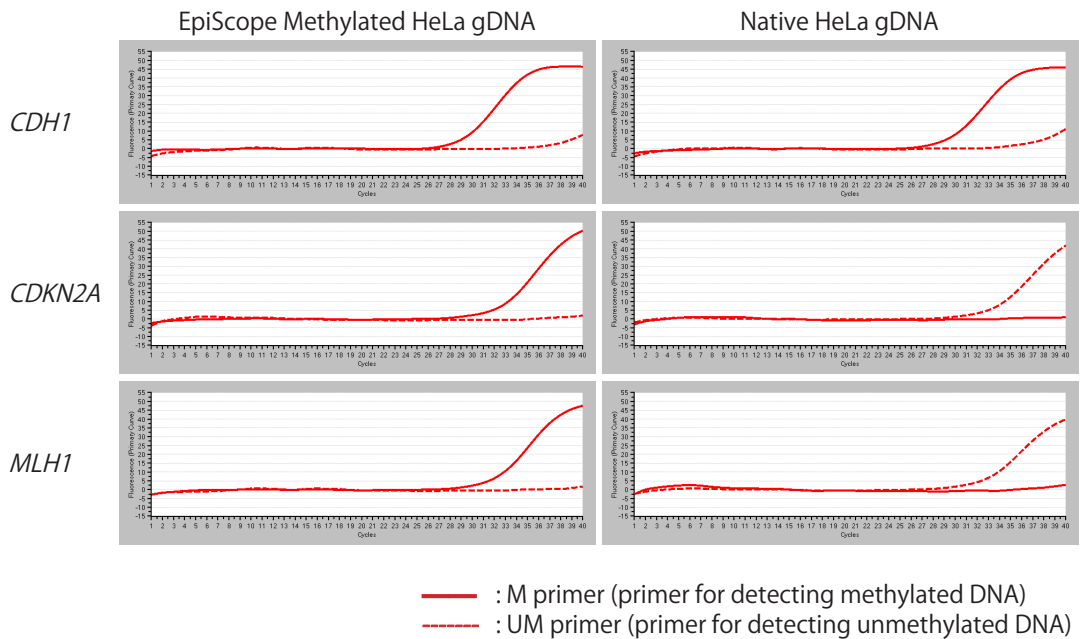


Figure 2. Detection with Thermal Cycler Dice Real Time System // (discontinued), a real-time PCR device

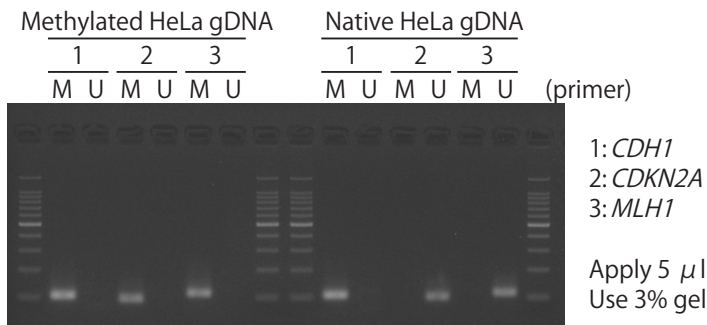


Figure 3. Endpoint detection

IX. Troubleshooting

This product's reaction system is designed to provide a high reactivity and to distinguish methylated/unmethylated DNA site. If the result is less than desirable, review the primer concentration or the PCR condition in accordance with the following procedure.

<No amplification, poor reactivity>

- Increase the primer concentration
- Lower the annealing temperature
- Increase the number of cycles up to 45

<Methylated/unmethylated indistinguishable, significant non-specific amplification>

- Lower the primer concentration
- Raise the annealing temperature
- Reduce the number of cycles

X. Related Products

EpiScope® Methylated HeLa gDNA (Cat. #3520)

EpiScope® Methylated HCT116 gDNA (Cat. #3522)

EpiScope® Unmethylated HCT116 DKO gDNA (Cat. #3521)

TaKaRa EpiTaq™ HS (for bisulfite-treated DNA) (Cat. #R110A/B)*1

Thermal Cycler Dice™ Real Time System III (Cat. #TP950/TP970/TP980/TP990)*2

*1 This is a DNA polymerase optimized for PCR amplifications using bisulfite-treated DNA containing uracil as template.

*2 Not available in all geographic locations. Check for availability in your area.

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Thermal Cycler Dice and EpiTaq are trademarks of Takara Bio Inc.

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