Cat. # R100A

For Research Use

TaKaRa

EpiScope® MSP Kit

Product Manual

v202202Da

Table of Contents

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.

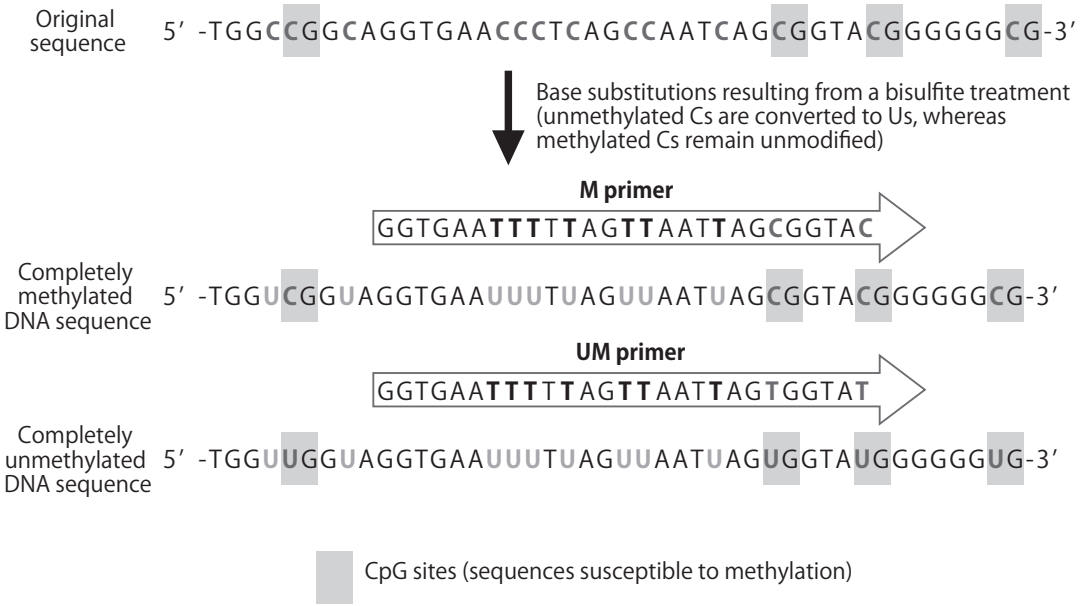


Figure 1. Principle of MSP

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 Mg^{2+} 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℃
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.

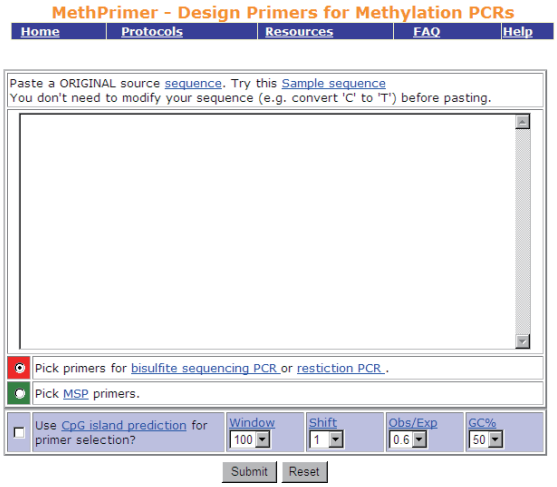
(2) Use fresh disposable tips to avoid any potential contamination between samples when preparing or dispensing reaction mixtures.

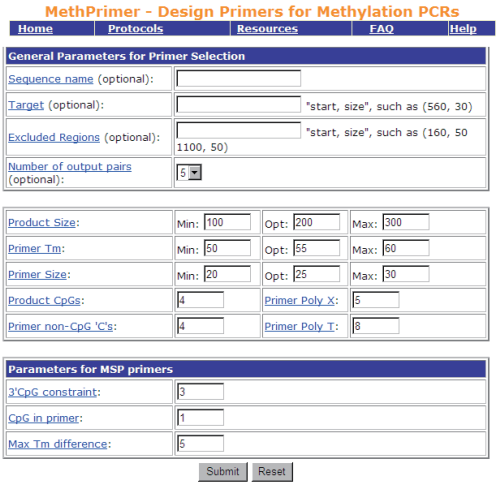
VII. Protocol

1. Primer design

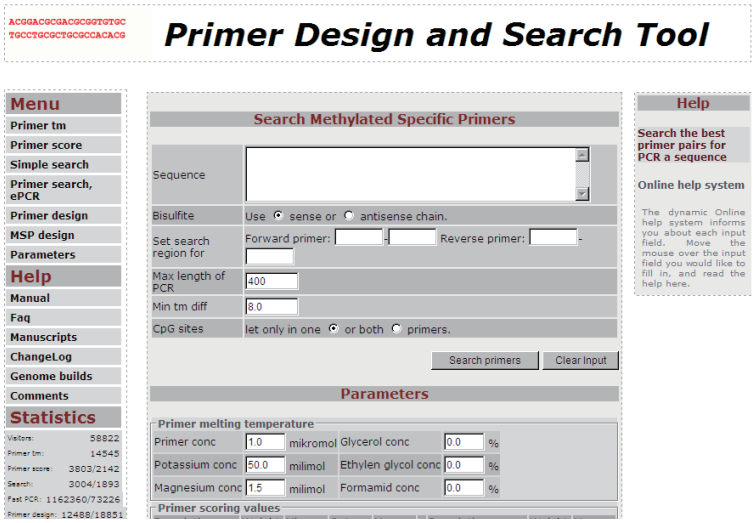
We recommend using a primer design tool that is specific for bisulfite-treated sequences. The following design tools are available online, free of charge. (For specific operating instructions, please refer to the help section of each tool.)
The optimum size of amplification products is between 80 and 150 bp (amplifications of up to 200 bp are possible).

MethPrimer
https://www.urogene.org/methprimer/index1.html





BiSearch
https://bisearch.enzim.hu/?m=msp



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℃ 30 sec
↓
98℃ 5 sec
55℃ 30 sec
72℃ 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℃ 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℃ 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.

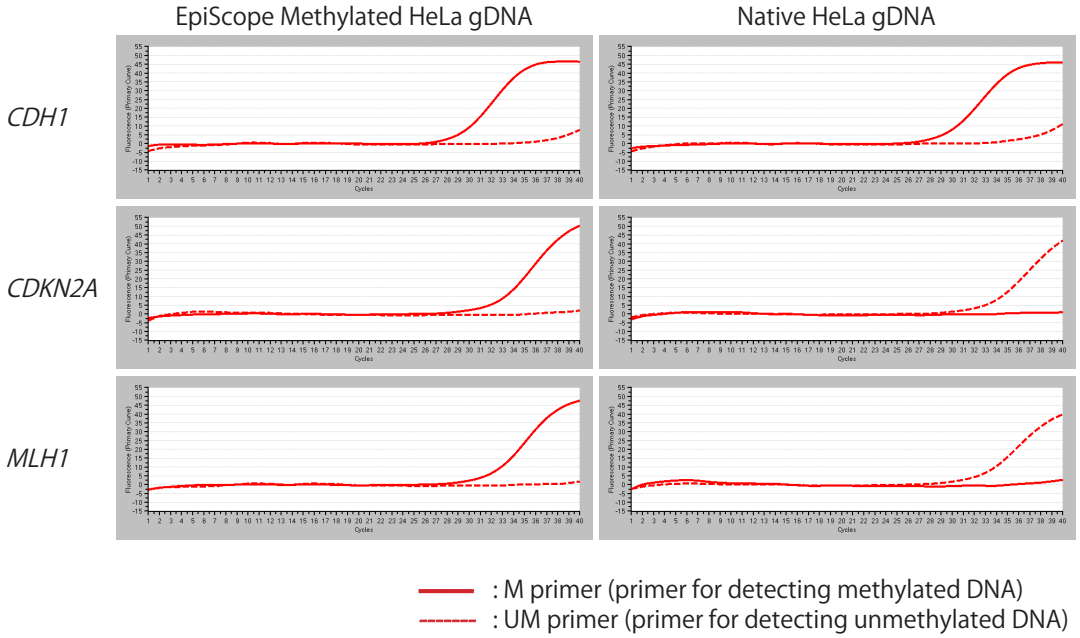


Figure 2. Detection with Thermal Cycler Dice Real Time System *II* (discontinued), a real-time PCR device

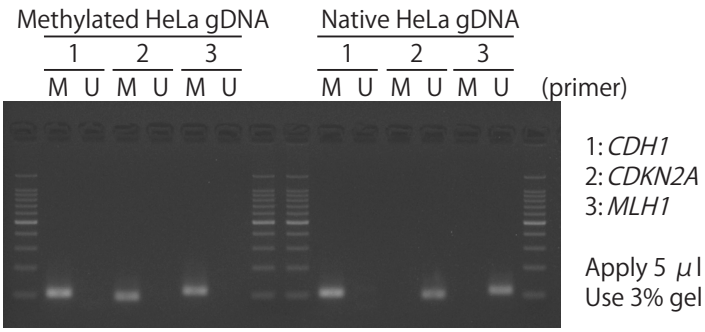


Figure 3. Endpoint detection

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.