

Cat. # R110A

For Research Use

TaKaRa

TaKaRa EpiTaq™ HS (for bisulfite-treated DNA)

Product Manual

v1111Da

Table of Contents

I. Description	3
II. Components	3
III. Storage	3
IV. Composition of PCR Mixture	3
V. PCR Conditions	4
VI. Electrophoresis of Amplification Products	4
VII. PCR Products.....	4
VIII. Primer Design	5
IX. Experimental Examples	6
X. Troubleshooting.....	7
XI. Related Product	8

I. Description

TaKaRa EpiTaq HS (for bisulfite-treated DNA) is a DNA polymerase optimized for PCR amplifications using bisulfite-treated DNA containing uracil as template. PCR reactions with bisulfite-treated DNA often pose particular technical challenges for conventional enzymes. TaKaRa EpiTaq HS and the reagents contained in this kit, however, allow adjustment of magnesium and dNTP concentrations to achieve optimal amplification efficiencies and specificities, facilitating excellent amplifications even for targets that previously were impossible to amplify.

TaKaRa EpiTaq HS is also a hot-start PCR enzyme with an anti-*Taq* antibody. Prior to high-temperature denaturation, the anti-*Taq* antibody suppresses polymerase activity, thereby preventing non-specific amplifications as a result of mispriming or primer dimer formation before starting PCR. This allows the use of conventional PCR conditions without requiring a special denaturation step.

II. Components

(1)	TaKaRa EpiTaq HS (5 U/ μ l)	250 U
(2)	10X EpiTaq PCR Buffer (Mg ²⁺ free)	1 ml
(3)	dNTP Mixture (2.5 mM each)	1.2 ml
(4)	25 mM MgCl ₂	1.2 ml

III. Storage -20°C

IV. Composition of PCR Mixture

	Volume	Final Concentration
TaKaRa EpiTaq HS (5 U/ μ l)	0.25 μ l	1.25 U / 50 μ l
10X EpiTaq PCR Buffer (Mg ²⁺ free)	5 μ l	1X
25 mM MgCl ₂	5 μ l	2.5 mM
dNTP Mixture (2.5 mM each)	6 μ l	0.3 mM
Template	< 100 ng	
Primer 1	20 pmol	0.4 μ M
Primer 2	20 pmol	0.4 μ M
Sterilized distilled water	to 50 μ l	

For a first trial, use the reaction mixture described above. If this reaction mixture fails to result in desired levels of product amplification or results in the appearance of extra bands, adjusting the concentration of MgCl₂, dNTP mixture, or primer may improve results. For details, see section X. Troubleshooting.

PCR reaction mixtures can be prepared at room temperature. Each component, however, should be kept on ice.

V. PCR Conditions

98°C	10 sec. *1	} 30 - 40 cycles
55°C	30 sec.	
72°C	30 sec. (~ 500 bp) or 1 min. (500 bp ~ 1 kb)*2	

- * 1: Select denaturation conditions compatible with the PCR thermal cycler and tubes used for the reaction. In general, treat at 98°C for 5 - 10 sec. or at 94°C for 20 - 30 sec.
- * 2: Set extension time according to the amplification size. In general, use 30 sec. for an amplification products of up to 500 bp, and 1 min. for amplification products ranging from 500 bp to 1 kb. As a general guideline, use 1 min. per 1 kb when the product size exceeds 1 kb. If template DNA was damaged during the bisulfite treatment process, amplification efficiency will decrease for larger products.

VI. Electrophoresis of Amplification Products

After the reaction is complete, add 6X Loading Buffer (supplied with TaKaRa DNA molecular weight markers) to an aliquot of the reaction mixture (5 - 10 μ l) in a ratio of 1:5 by volume, and analyze using agarose gel electrophoresis. After electrophoresis, stain the gel using 1.0 μ g/ml of ethidium bromide for 20 - 30 min. Visualize DNA bands under UV irradiation.

VII. PCR Products

The majority of PCR products amplified using TaKaRa EpiTaq HS have a single A nucleotide added at 3' termini, allowing the PCR products to be cloned directly into T-vectors such as pMD20 (Cat. #3270) or pMD19 (Simple) (Cat. #3271).

These PCR products may also be used for blunt-end cloning after blunting and phosphorylation.

VIII. Primer Design

When designing primers for bisulfite-treated DNA, the use of a primer design tool specifically created for this purpose is recommended. The following design tools are available online free of charge. (For specific operating procedures, please refer to each web application.)

To ensure successful amplification with bisulfite-treated DNA as template, an amplification product length of no more than 300 bp is recommended. At Takara Bio, amplifications of up to approximately 1 kb have been achieved. (See section IX. Experimental Examples.)

MethPrimer

<http://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

Primer Design and Search Tool

Menu
Primer tm
Primer score
Simple search
Primer search, ePCR
Primer design
MSP design
Parameters
Help
Manual
FAQ
Manuscripts
Chromatogram
Genome Builds
Comments
Statistics

Statistics
Size: 58920
Size tm: 14540
Primer score: 2003/2142
Search: 2004/1892
Path ID: 1142340/72224
Primer setp: 12488/1892

Search Methylated Specific Primers

Sequence:

Bisulfite: Use sense or antisense chain.

Set search region for: Forward primer: Reverse primer:

Max length of PCR:

Min tm diff:

CpG sites: set 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: 2.0 mmolol Ethylen glycol conc: 0.0 %

Magnesium conc: 1.5 mmolol 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. Mouse the mouse over the input field you would like to edit, and read the help page.

BiSearch

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

MethPrimer - Design Primers for Methylation PCRs

Home Protocols Resources FAQ Help

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)
1100, 50

Number of output pairs (optional):

Product Size:	Min: <input type="text" value="100"/>	Opt: <input type="text" value="200"/>	Max: <input type="text" value="300"/>
Primer Tm:	Min: <input type="text" value="50"/>	Opt: <input type="text" value="55"/>	Max: <input type="text" value="60"/>
Primer Size:	Min: <input type="text" value="20"/>	Opt: <input type="text" value="25"/>	Max: <input type="text" value="30"/>
Product CpGs:	<input type="text" value="4"/>	Primer Poly X:	<input type="text" value="5"/>
Primer non-CpG 'C's:	<input type="text" value="4"/>	Primer Poly T:	<input type="text" value="8"/>

IX. Experimental Examples

PCR amplification was performed with bisulfite-treated HeLa genomic DNA as template.

[Method]

Bisulfite treatment: MethylEasy™ Xceed Rapid DNA Bisulfite Modification Kit (Cat. #ME002)
 Target: CpG island region upstream of *CDH1*, *MLH1*, or *BRCA1* genes
 Amplification size: 153 bp (*CDH1*), 297 bp (*CDH1*), 136 bp (*MLH1*), 292 bp (*MLH1*), 613 bp (*BRCA1*), 1,017 bp (*BRCA1*)

<Reaction mixture composition>

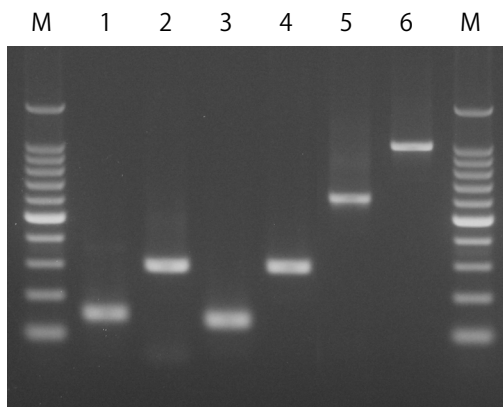
	Volume	Final Concentration
Bisulfite-treated HeLa genomic DNA (50 ng/μl)	2 μl	100 ng / 50 μl
10X EpiTaq PCR Buffer (Mg ²⁺ free)	5 μl	1 ×
25 mM MgCl ₂	5 μl	2.5 mM
dNTP Mixture (2.5 mM each)	6 μl	0.3 mM
Sense Primer (20 μM)	1 μl	0.4 μM
Antisense Primer (20 μM)	1 μl	0.4 μM
TaKaRa EpiTaq HS (5 U/μl)	0.25 μl	1.25 U / 50 μl
Sterile distilled water	to 50 μl	

<PCR Conditions>

98°C 10 sec.
 55°C 30 sec.
 72°C [30 sec. for product <500 bp,
 1 min. for products ≥ 500 bp]

40 cycles

[Result]



Lane: Size: Gene:
 1: 153 bp (*CDH1*)
 2: 297 bp (*CDH1*)
 3: 136 bp (*MLH1*)
 4: 292 bp (*MLH1*)
 5: 613 bp (*BRCA1*)
 6: 1,017 bp (*BRCA1*)
 M: 100 bp DNA Ladder

Agarose: Agarose L03 (Cat. #5003)
 Gel concentration: 2%
 Stain: Ethidium bromide

Figure 1. PCR with bisulfite-treated HeLa genomic DNA as template

X. Troubleshooting

Event	Possible causes	Action
No amplification or poor amplification efficiency	Mg ²⁺ concentration	Raise the Mg ²⁺ concentration; try 2.5 - 3 mM. (See Figure 2)
	dNTP concentration	Lower the dNTP concentration; try 0.2 - 0.3 mM
	Annealing temperature	Try lowering temperature from the default of 55°C. Lower by 2°C per trial.
	Extension time	Increase the extension time from 30 sec. to 1 min. or from 1 to 2 min.
	Primer concentration	Raise the primer concentration; try 0.4 - 1 μM.
	Template fragmentation occurred during bisulfite treatment of DNA	Re-prepare bisulfite-treated template DNA.
Electrophoresis analysis shows smeared band(s) or extra band(s)	Mg ²⁺ concentration	Lower the Mg ²⁺ concentration; try 2 - 2.5 mM
	dNTP concentration	Raise the dNTP concentration; try 0.3 - 0.4 mM.
	Annealing temperature	Try raising temperature from the default of 55°C. Raise by 2°C per trial.
	Primer concentration	Lower the primer concentration; try 0.2 - 0.4 μM.

* Lowering the Mg²⁺ concentration increases specificity, while raising the Mg²⁺ concentration improves amplification efficiency and extension. Raising the dNTP concentration increases specificity, while lowering the dNTP concentration improves amplification efficiency and extension.

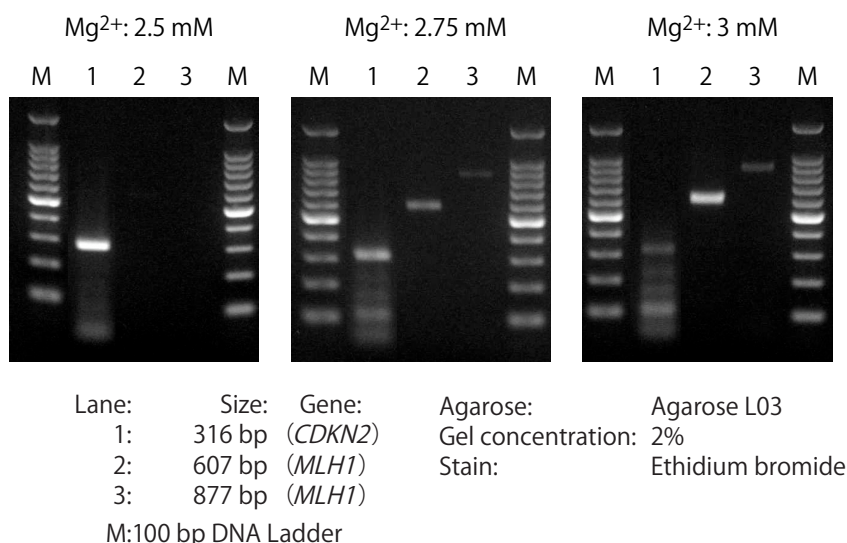


Figure 2. Improvements achieved by altering Mg²⁺ concentration

XI. Related Product

MethylEasy™ *Xceed* Rapid DNA Bisulphite Modification Kit (Cat. #ME002)

NOTICE TO PURCHASER : LIMITED LICENSE

[L15] Hot Start PCR

Licensed under U.S. Patent No. 5,338,671 and 5,587,287 and corresponding patents in other countries.

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