April 2008

EpiTect® PCR Control DNA Handbook

For performing 100 control reactions for methylation analysis using Human control DNA

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Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

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Kit Contents

EpiTect PCR Control DNA Set	**(100)**
Catalog no.	**59695**
Number of preps	**100**
Methylated human control DNA (bisulfite converted), 10 ng/µl	100 µl
Unmethylated human control DNA (bisulfite converted), 10 ng/µl	100 µl
Unmethylated human control DNA, 10 ng/µl	100 µl
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Shipping and Storage

The EpiTect PCR Control DNA Set is shipped at room temperature. The kit should be stored immediately upon receipt at –20°C in a constant-temperature freezer. When stored under these conditions and handled correctly, this product can be kept for at least 6 months without showing any reduction in performance.

As with any DNA, repeated freeze-thaw cycles should be avoided as this can lead to degradation of DNA.

Product Use Limitations

The EpiTect PCR Control DNA Set is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the EpiTect PCR Control DNA Set or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/ts/msds.asp where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of EpiTect PCR Control DNA Set is tested against predetermined specifications to ensure consistent product quality.

Introduction

Control reactions should be performed when undertaking methylation analysis, for example, when performing methylation-specific PCR (MSP), to ensure that the PCR primers are specific for the detection of methylated or unmethylated bisulfite converted DNA.

To perform control reactions, methylated bisulfite converted DNA, unmethylated bisulfite converted DNA, and genomic DNA are required. Each type of DNA should be used to show the specificity of the PCR. For example, the primer specific for methylated DNA will only show a signal for the methylated control DNA (see Table 5). In addition, genomic DNA can be used to determine the bisulfite conversion efficiency of bisulfite reactions.

The EpiTect PCR Control DNA Set consists of three different types of human DNA:

- Amplified human genomic DNA (completely unmethylated)
- Completely unmethylated human genomic DNA — bisulfite converted
- Completely methylated human genomic DNA — bisulfite converted

Complete in vitro methylation of the control DNA was achieved using SssI methylase. Bisulfite conversion of control DNA was achieved using the EpiTect Bisulfite Kit.

Each control DNA is stored in Buffer EB in a convenient, ready-to-use 10 ng/µl solution.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- Reaction tubes
- Pipets and pipet tips (aerosol resistant)
- Thermal cycler
- Mineral oil (only if thermal cycler does not have a heated lid)
- Primers should be purchased from an established oligonucleotide manufacturer, such as Operon Biotechnologies (www.operon.com). Lyophilized primers should be dissolved in TE to provide a stock solution of 100 µM; concentration should be checked by spectrophotometry. Primer stock solutions should be stored in aliquots at –20°C.
- PCR reagents (e.g., EpiTect MSP Kit)

Protocol: Using the EpiTect PCR Control DNA Set

Important points before starting

- Set up all reaction mixtures in an area separate from those used for DNA preparation or PCR product analysis.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.
- We recommend use of 10 ng template DNA in each PCR
- Control DNA can be diluted in an appropriate buffer (e.g., TE or water).

  Note: This may affect the stability of the DNA during storage.

Procedure

1. **Setup of PCR Control Reactions**

   Use 1 µl (10 ng) of each control DNA for every PCR reaction.

   We recommend using the EpiTect MSP Kit for highly specific and reliable methylation-specific PCR results.

Table 1. Example Reaction Setup Using EpiTect MSP Master Mix

Component	Volume/reaction	Final concentration
EpiTect Master Mix, 2x	25 µl	1x
Diluted primer mix		
Primer A*	variable	0.3–0.4 µM
Primer B*	variable	0.3–0.4 µM
Template DNA		
Template DNA	1 µl	10 ng
RNAse-free water	variable	
Total volume	**50 µl**	

Note: If smaller or larger reaction volumes are used, adjust the amount of each component accordingly.

* A final primer concentration of 0.3–0.4 µM is optimal for most applications. However, for individual determination of best concentration, a primer titration from 0.2 µM to 0.5 µM can be performed.

Table 2. Cycling conditions for using EpiTect MSP Master Mix

			Additional comments
Initial activation step:	**10 min**	**95°C**	The HotStarTaq d-Tect Polymerase is activated by this heating step
3-Step cycling			
Denaturing:	15 s	94°C	
Annealing:	30 s	50–55°C	Approximately 8°C below T_m of primers
Extension:	30 s	**72°C**	For PCR products longer than 500 bp, use an extension time of 60 s/500 bp
Number of cycles:	30–40		
Final extension:	10 min	**72°C**	

Table 3. Reaction Setup Using EpiTect MethyLight PCR Kit

Component	Volume/reaction	Final concentration
EpiTect MethyLight Master Mix, 2x	25 µl	1x
Diluted primer mix		
Primer A*	variable	0.4 µM
Primer B*	variable	0.4 µM
Probe 1 (methylated)	variable	0.2 µM
Probe 2 (unmethylated)	variable	0.2 µM
Template DNA		
Template DNA	1 µl	10 ng
RNase-free Water	variable	-
Total volume	**50 µl**	

Note: If smaller or larger reaction volumes are used, adjust the amount of each component accordingly.

* A final primer concentration of 0.4 µM is optimal for most applications. However, for individual determination of best concentration, a primer titration from 0.4 µM to 0.6 µM can be performed.

Table 4. Cycling conditions for methylation PCR analysis using TaqMan probes

Step	Time	Temperature	Additional comments
Initial PCR activation step	**5 min**	**95°C**	HotStarTaq *Plus* DNA Polymerase is activated by this heating step
2-step cycling:			**Important: Optimal performance is only assured using these cycling conditions**
Denaturation	**15 s**	**95°C**	
Annealing/Extension	**60 s**	**60°C**	Combined annealing/extension step with fluorescence data collection
Number of cycles	40–45		The number of cycles depends on the amount of template DNA

2. Check the specificity of primers and PCR conditions

The expected results when using control DNA and particular primer pairs are shown in Table 5 below.

Table 5. Expected PCR Results

Type of DNA	Primer for unmethylated target gene PCR 1	Primer for unmethylated target gene (bisulfite converted) PCR 2	Primer for methylated target gene (bisulfite converted) PCR 3
Unmethylated control DNA	**PCR product**	No PCR product	No PCR product
Unmethylated control DNA (bisulfite converted)	No PCR product	**PCR product**	No PCR product
Methylated control DNA (bisulfite converted)	No PCR product	No PCR product	**PCR product**
No template control	No PCR product	No PCR product	No PCR product

3. Check the sensitivity of primers and PCR conditions

Depending on the method of detection, primers should be chosen so that the corresponding PCR products can be easily distinguished from one another, for example by size difference.

The sensitivity of the MSP assay can be assessed by using both primers specific for methylated DNA and primers specific for unmethylated DNA with different amounts of control DNA (Table 6). The control DNA should be diluted in TE where necessary.

Table 6. Determining MSP sensitivity.

Type of DNA	Amount of control DNA							
	PCR 1	PCR 2	PCR 3	PCR 4	PCR 5	PCR 6	PCR 7	PCR 8
Unmethylated control DNA (bisulfite converted)	10 ng	9.9 ng	9 ng	5 ng	1 ng	0.1 ng	0 ng	0 ng
Methylated control DNA (bisulfite converted)	0 ng	0.1 ng	1 ng	5 ng	9 ng	9.9 ng	10 ng	0 ng

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx . The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

		Comments and suggestions
no product		
a)	Pipetting error or missing reagent	Repeat the PCR. Check the concentrations and storage conditions of reagents, including primers.
b)	Primer concentration not optimal or primers degraded	Repeat the PCR with different primer concentrations from 0.2–0.5 µM of each primer (in 0.1 µM steps). In particular, when performing highly sensitive PCR, check for possible degradation of the primers on a denaturing polyacrylamide gel*.
c)	Problems with starting template	Check the concentration, storage conditions, and quality of the starting template. If necessary, make new serial dilutions of template nucleic acid from stock solutions. Repeat the PCR using the new dilutions.
d)	no product	Please see Table 5, page 11: Expected PCR Results.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs) available from the product supplier.

Appendix A: Specificity and Reliability

To confirm PCR specificity and reliability when using the EpiTect PCR Control DNA set, a 4-plex PCR was performed.

The QIAGEN Multiplex PCR Kit was used according to the protocol in the handbook. Four primer pairs were used in parallel on 5 ng of each control DNA (Figure 1):

- Primer pair 1: SYR — detection of genomic DNA that has not been bisulfite treated (expected fragment size: 76 bp)
- Primer pair 2: βActin — detection of bisulfite converted genomic DNA (expected fragment size: 536 bp)
- Primer pair 3: VHL — detection of unmethylated and bisulfite treated genomic DNA (expected fragment size: 165 bp)
- Primer pair 4: CDH3 — detection of methylated and bisulfite treated genomic DNA (expected fragment size: 101 bp)

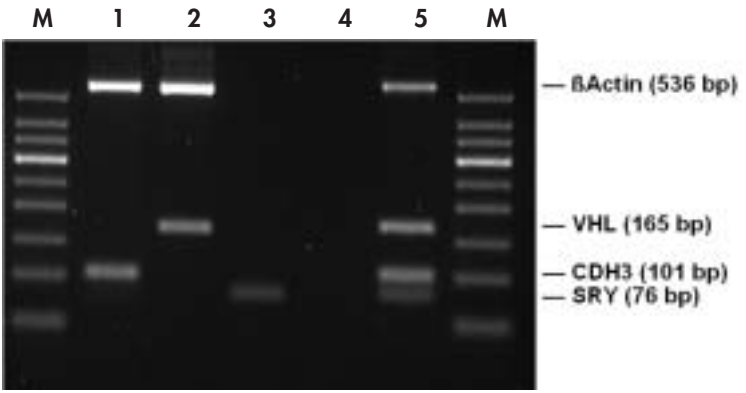

Figure 1. Specificity and reliability results. 10 µl each PCR reaction (described above) was run on a 2% agarose gel. **1**: methylated, bisulfite converted DNA; **2**: unmethylated, bisulfite converted DNA; **3**: genomic DNA (not bisulfite converted); **4**: no template control; **5**: Mixture of all DNA from lanes 1, 2, and 3. **M**: GelPilot 50 bp Ladder.

Ordering Information

Product	Contents	Cat. no.
Related products		
EpiTect Bisulfite Kits — for complete bisulfite conversion and cleanup of DNA for methylation analysis		
EpiTect Bisulfite Kit (48)	48 EpiTect Bisulfite Spin Columns, Reaction Mix, DNA Protect Buffer, Carrier RNA, Buffers	59104
EpiTect 96 Bisulfite Kit (2)	2x EpiTect Bisulfite 96-well Plates, Reaction Mix, DNA Protect Buffer, Carrier RNA, Buffers	59110
EpiTect Control DNA — for evaluation of PCR primers used for methylation analysis		
EpiTect Control DNA, methylated (100)	Methylated and bisulfite converted human control DNA for 100 control PCRs	59655
EpiTect Control DNA, unmethylated (100)	Unmethylated and bisulfite converted human control DNA for 100 control PCRs	59665
EpiTect Control DNA (1000)	Unmethylated human control DNA for 1000 control PCRs	59568
EpiTect MSP Kit — for highly accurate methylation-specific. PCR without optimization		
EpiTect MSP Kit (25)	EpiTect MSP Master Mix for 25 x 50 µl reactions	59303
EpiTect MSP Kit (100)	EpiTect MSP Master Mix for 100 x 50 µl reactions	59305
EpiTect MSP Kit (400)	EpiTect MSP Master Mix for 400 x 50 µl reactions	59307

Ordering Information

Product	Contents	Cat. no.
EpiTect Whole Bisulfitome Kit — for amplification of bisulfite converted DNA		
EpiTect Whole Bisulfitome Kit (25)	REPLI-g Midi DNA Polymerase, EpiTect WBA Reaction Buffer, nuclease-free water for 25 whole bisulfitome amplification reactions	59203
EpiTect Whole Bisulfitome Kit (100)	REPLI-g Midi DNA Polymerase, EpiTect WBA Reaction Buffer, nuclease-free water for 100 whole bisulfitome amplification reactions	59205
EpiTect MethyLight PCR Kit — for real-time quantification of methylation status		
EpiTect MethyLight PCR Kit (200)	Master Mix for methylation-specific real-time PCR analysis, 200 x 50 µl reactions	59436
EpiTect MethyLight PCR Kit (1000)	Master Mix for methylation-specific real-time PCR analysis, 1000 x 50 µl reactions	59438
EpiTect MethyLight PCR + ROX Vial Kit (200)	Master Mix without ROX for methylation-specific real-time PCR analysis, 200 x 50 µl reactions	59496
EpiTect MethyLight PCR + ROX Vial Kit (1000)	Master Mix without ROX for methylation-specific real-time PCR analysis, 1000 x 50 µl reactions	59498

All Kits are intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Notes

Notes

Limited License Agreement

Use of this product signifies the agreement of any purchaser or user of the EpiTect PCR Control DNA Set to the following terms:

1. The EpiTect PCR Control DNA Set may be used solely in accordance with the EpiTect PCR Control DNA Handbook and for use with components contained in the Kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this Kit with any components not included within this Kit except as described in the EpiTect PCR Control DNA Handbook and additional protocols available at www.qiagen.com .
2. Other than expressly stated licenses, QIAGEN makes no warranty that this Kit and/or its use(s) do not infringe the rights of third-parties.
3. This Kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the Kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the Kit and/or its components.

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