# **EpiTect® MSP Handbook**

For highly accurate methylation-specific PCR without optimization



### **QIAGEN Sample and Assay Technologies**

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

#### QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit <a href="https://www.giagen.com">www.giagen.com</a>.



QIAGEN is a member of the Forest Stewardship Council (FSC). For the production of printed materials, including handbooks, QIAGEN has a policy to select suppliers that comply with FSC standards for printing processes and well-managed forests.

### **Contents**

Kit Contents	4
Shipping and Storage	4
Product Use Limitations	4
Product Warranty and Satisfaction Guarantee	4
Technical Assistance	5
Safety Information	5
Quality Control	6
Product Specifications	7
Introduction	8
Equipment and Reagents to Be Supplied by User	10
Protocol	
PCR Using the EpiTect MSP Kit	11
Troubleshooting Guide	13
Ordering Information	17

### **Kit Contents**

EpiTect MSP Kit	(25)	(100)	(400)
Catalog no.	59303	59305	59307
Number of 50 µl reactions	25	100	400
EpiTect MSP Master Mix	1 x 850 µl	3 x 850 µl	12 x 850 µl
RNase-Free Water	1 x 1.9 ml	$2 \times 1.9 \text{ ml}$	8 x 1.9 ml
Handbook	1	1	1

# **Shipping and Storage**

The EpiTect MSP Kit is shipped on dry ice. The kit should be stored immediately upon receipt at -30 to  $-15^{\circ}$ C in a constant-temperature freezer. When stored under these conditions and handled correctly, this product can be stored at least until the expiration date (see the inside of the kit lid) without showing any reduction in performance.

### **Product Use Limitations**

The EpiTect MSP Kit is intended for molecular biology applications. This product is not intended 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 MSP Kit 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 <a href="www.qiagen.com/Support">www.qiagen.com/Support</a> or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit <a href="www.qiagen.com">www.qiagen.com</a>).

# **Safety Information**

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at <a href="https://www.qiagen.com/safety">www.qiagen.com/safety</a> where you can find, view, and print the SDS for each QIAGEN kit and kit component.

# **Quality Control**

#### **Enzyme:**

Unit assay: Sonicated herring sperm DNA (12.5 µg) is

incubated with 0.01–0.1 units of HotStarTaq® d-Tect Polymerase in assay buffer (25 mM TAPS [tris (hydroxymethyl)-methyl-amino-propane-sulfonic acid, sodium salt], pH 9.3 at 20°C; 50 mM KCl; 2 mM MgCl $_2$ ; 1 mM DTT; 200  $\mu$ M of each dNTP; 100  $\mu$ Ci [a- $^{32}$ P] dCTP) at 72°C for 30 min. The amount of incorporated dNTPs is determined by precipitation with trichloroacetic acid. HotStarTaq d-Tect Polymerase is activated by heating for 3 h at 80°C prior to activity

measurement.

Amplification efficiency assay: The amplification efficiency is tested in parallel

amplification reactions and is indicated under

"Amp".

PCR reproducibility assay: PCR reproducibility and specificity are tested in

parallel amplification reactions. The reactions

must yield a single specific product.

Exonuclease activity assay: Linearized plasmid DNA is incubated with

HotStarTaq d-Tect Polymerase in PCR Buffer. Exonuclease activity per unit of enzyme is

indicated under "Exo".

Endonuclease activity assay: Plasmid DNA is incubated with HotStarTag d-Tect

Polymerase in PCR Buffer. Endonuclease activity per unit of enzyme is indicated under "Endo".

RNase activity assay: RNA is incubated with HotStarTaq d-Tect

Polymerase in PCR Buffer. RNase activity per unit of enzyme is indicated under "RNase".

Protease activity assay: HotStarTaq d-Tect Polymerase is incubated in

storage buffer. Protease activity per unit of

enzyme is indicated under "Protease".

Self-priming activity assay: Assays are performed under standard PCR

conditions, without primers, HotStarTaq d-Tect Polymerase and human genomic DNA (purified with the QIAamp® DNA Blood Mini Kit). The absence of PCR product is indicated by "No"

under "Self priming".

#### **EpiTect MSP Master Mix Kit:**

PCR reproducibility assay: The PCR reproducibility assay described above is

performed in parallel using EpiTect MSP Master Mix and using the separate reagents with the same

lot numbers.

## **Product Specifications**

#### **Enzyme:**

HotStarTaq d-Tect Polymerase is a modified form of a recombinant 94 kDa DNA polymerase, originally isolated from *Thermus aquaticus*, cloned in *E. coli*. (Deoxynucleoside-triphosphate: DNA deoxynucleotidyltransferase, EC 2.7.7.7).

5'-3' exonuclease activity: Yes
3'-5' exonuclease activity: No
Nuclease contamination: No
Protease contamination: No

RNase contamination: No Self-priming activity: No

**Buffers and reagents:** 

EpiTect MSP Master Mix: 2x concentrated. Contains HotStarTaq

d-Tect Polymerase, Tris·Cl, KCl,  $(NH_4)_2SO_4$ ,

MgCl<sub>2</sub>, and dNTPs.

### Introduction

Methylation-specific PCR (MSP) is a particularly demanding application as, in order to provide reliable results, it requires high specificity to discriminate between cytosine and thymine bases derived from methylated and unmethylated cytosines following bisulfite conversion.

HotStarTaq d-Tect Polymerase is a modified form of the recombinant 94 kDa *Taq* DNA Polymerase from QIAGEN. The modification prevents elongation of mismatched bases at the 3' end of the primer. This allows discrimination of single base mismatches during primer annealing and extension. This increases the potential primer sites for assay development and allows simplified primer design for methylation analysis.

HotStarTaq d-Tect Polymerase is provided in an inactive state with no polymerase activity at ambient temperatures. This prevents the formation of misprimed products and primer–dimers at low temperatures. HotStarTaq d-Tect Polymerase is activated by a 10-minute, 95°C incubation step, which can easily be incorporated into existing thermal cycling programs. HotStarTaq d-Tect Polymerase provides high PCR specificity. PCR setup is quick and convenient as all reaction components can be combined at room temperature.

#### **QIAGEN EpiTect PCR Buffer**

The new QIAGEN EpiTect PCR Buffer facilitates amplification of specific PCR products with significantly increased primer binding specificity. Based on the original QIAGEN PCR Buffer, this new formulation enables a high ratio of specific to nonspecific primer binding during the annealing step of every PCR cycle. Owing to a uniquely balanced combination of KCl and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, the PCR buffer provides stringent primerannealing conditions over a wider range of annealing temperatures. Optimization of PCR by varying the annealing temperature or the Mg<sup>2+</sup> concentration is dramatically reduced and often not required.

### **EpiTect MSP Master Mix Kit**

Benefits of the EpiTect MSP Master Mix Kit include reduced number of pipetting steps and reduced risk of contamination. There is no need for reaction optimization, thereby reducing the need for validation and, therefore, saving time.

### Specificity and sensitivity

HotStarTaq d-Tect Polymerase, in combination with the new QIAGEN EpiTect PCR Buffer, with its balanced potassium and sodium salts, promotes specific primer–template annealing and simultaneously reduces nonspecific annealing. Maximum yields of specific products are obtained even when using extremely low template amounts.

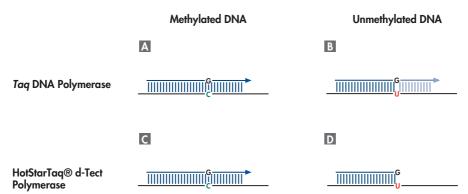


Figure 1. Increased mismatch discrimination of HotStarTaq d-Tect Polymerase. During the annealing of PCR primers, methylation-specific primers also often bind to the unmethylated converted DNA with a mismatch or several mismatches at the 3' end of the primer (B). Taq DNA Polymerase can efficiently elongate these primers regardless of the primer mismatch (A and B). Due to its increased ability to discriminate between mismatches, genetically engineered HotStarTaq d-Tect Polymerase recognizes a mismatch at the 3' end of the methylation-specific primer and therefore prevents primer extension. (C) HotStarTaq d-Tect Polymerase only elongates primers without mismatch. (D) Thus, HotStarTaq d-Tect Polymerase increases reliability in MSP PCR reactions and prevents false-positive amplification reactions, facilitating primer design.

### Control reactions

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

To perform control reactions, methylated bisulfite converted DNA, unmethylated bisulfite converted DNA, and genomic DNA are required. In addition, genomic DNA can be used to determine the bisulfite conversion efficiency of bisulfite reactions. We recommend use of EpiTect PCR Control DNA (see Ordering Information, page 17). Each EpiTect PCR 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 safety data sheets (SDSs), 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 (<u>www.operon.com</u>). 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.

# Protocol: PCR Using the EpiTect MSP Kit

#### Important points before starting

- EpiTect Master Mix requires an activation step of 10 min at 95°C (see step 6 of this protocol).
- EpiTect Master Mix provides an optimized concentration of MgCl<sub>2</sub> in the final reaction mix, which will produce satisfactory results in most cases. However, if a higher Mg<sup>2+</sup> concentration is required, prepare a stock solution containing 25 mM MgCl<sub>2</sub>.
- 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.

#### **Procedure**

- Thaw the EpiTect Master Mix, nucleic acid template, primer solutions, and RNase-free water. Mix well before use.
- 2. Mix the EpiTect Master Mix by vortexing briefly and dispense 25 µl into each PCR tube according to Table 1.

It is important to mix the EpiTect Master Mix before use in order to avoid localized concentrations of salt. It is not necessary to keep reaction vessels on ice since HotStarTag d-Tect Polymerase is inactive at room temperature.

Table 1. Reaction composition 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 added at step 4	variable	$<$ 200 ng/50 $\mu$ l reaction
RNase-free water	variable	
Total volume	50 µl	

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

- 3. Add the appropriate volume of the diluted primer mix into the PCR tubes containing the master mix.
- 4. Add template DNA (<200 ng/50 µl reaction) to the individual PCR tubes.
- 5. When using a thermal cycler with a heated lid, do not use mineral oil. Proceed directly to step 6. Otherwise, overlay with approximately 50 µl mineral oil.
- 6. Program the thermal cycler according to the manufacturer's instructions.

**Note**: Each PCR program must start with an initial heat activation step at 95°C for 10 min.

A typical PCR cycling program is outlined in Table 2. For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.

Table 2. Optimized cycling protocol

			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 $T_{\rm m}$ 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	

### 7. Place the PCR tubes in the thermal cycler and start the cycling program.

**Note**: After amplification, samples can be stored overnight at  $2-8^{\circ}\text{C}$  or at  $-20^{\circ}\text{C}$  for longer storage.

## **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: <a href="www.qiagen.com/FAQ/FAQList.aspx">www.qiagen.com/FAQ/FAQList.aspx</a>. 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 <a href="www.qiagen.com">www.qiagen.com</a>).

#### Comments and suggestions

Ordering Information, page 17).

a)	Incorrect primer design	Check primer design. Note that after bisulfite treatment of DNA, both strands are no longer complementary. Therefore, both primers have to be redesigned to match either the forward or the reverse strand.
		We recommend checking PCR specificity and reliability with EpiTect PCR Control DNA (see

b) Pipetting error or missing reagent

Little or no product

Repeat the PCR. Check the concentrations and storage conditions of reagents, including primers. Ensure a 1:1 ratio of EpiTect MSP Master Mix to primer–template solution is maintained.

c) HotStarTaq d-TectPolymerase not activated

Check whether PCR was started with an initial incubation step at 95°C for 10 min.

An activation time of less than 10 min may lead to insufficient reactivation of HotStarTaq d-Tect Polymerase.

d) Incorrect annealing temperature or time

Decrease annealing temperature in  $2^{\circ}C$  steps. Annealing time should be 30 seconds. Difficulties in determining the optimal annealing temperature can be overcome in many cases by performing touchdown PCR.

 e) Primer concentration not optimal or primers degraded Repeat the PCR with different primer concentrations from  $0.1-0.5~\mu\text{M}$  of each primer (in  $0.1~\mu\text{M}$  steps). In particular, when performing highly sensitive PCR, check for possible degradation of the primers on a denaturing polyacrylamide gel.\*

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

### Comments and suggestions

f)	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.
g)	Mg <sup>2+</sup> concentration not optimal	Perform PCR with different final concentrations of Mg <sup>2+</sup> . Add additional Mg <sup>2+</sup> in 0.25 mM steps using a 25 mM MgCl <sub>2</sub> solution.
h)	Insufficient number of cycles	Increase the number of cycles in steps of 5 cycles
i)	Extension time too short	Increase the extension time in increments of 30 s. For PCR using genomic DNA, increase the concentration of genomic DNA in the reaction.
i)	Incorrect denaturation temperature or time	Denaturation should be at 94°C for 15 s. Ensure that the initial 10 min 95°C incubation step was performed as described in step 6 of the PCR protocol (page 12).
k)	Insufficient starting template	Perform a second round of PCR using a nested PCR approach.
l)	Chosen fragment size is too big	Choose amplicon fragment size of up to 700 bp. We recommend using the EpiTect Bisulfite Kit for conversion, in order to get PCR fragments of appropriate size.
		With conversion kits from different suppliers, the fragment sizes might be much less than 700 bp.
m)	PCR overlaid with mineral oil when using a thermal cycler with a heated lid	When performing PCR in a thermal cycler with a heated lid, do not overlay the PCR samples with mineral oil if the heated lid is switched on as this may decrease the yield of PCR product.
n)	Problems with the thermal cycler	Check the power to the thermal cycler and that the thermal cycler has been correctly programmed.
0)	PCR tubes or plates do not fit in the cycler block perfectly.	Use PCR tubes or plates recommended by the thermal cycler manufacturer.

#### Comments and suggestions

#### Product is multi-banded

a) HotStarTaq d-Tect
 Polymerase activation
 time too long

Check whether PCR was started with an initial incubation step at 95°C for only 10 min.

b) Annealing temperature too low

An activation time greater than 10 min may lead to primer-dimer formation or multibanded products.

 Primer concentration not optimal or primers degraded Increase annealing temperature in 2°C steps. Annealing time should be 30 seconds. Difficulties in determining the optimal annealing temperature can be overcome in many cases by performing touchdown PCR.

Repeat the PCR with different primer concentrations

(from 0.1–0.5  $\mu M$  of each primer in 0.1  $\mu M$  steps). In particular, when performing highly sensitive PCR check for possible degradation of the primers on a denaturing polyacrylamide gel.\*

d) Primer design

Review primer design. Following bisulfite treatment of DNA, both strands are no longer complementary. Therefore, both primers have to be redesigned to match either the forward or the reverse strand.

We recommend checking PCR specificity and reliability with EpiTect PCR Control DNA (see Ordering Information, page 17).

#### Product is smeared

Too much starting template

Check the concentration and storage conditions of the starting template. Make serial dilutions of template nucleic acid from stock solutions. Perform PCR using these serial dilutions.

b) Carryover contamination

If the negative-control PCR (without template DNA) shows a PCR product or a smear, exchange all reagents. Use disposable pipet tips containing hydrophobic filters to minimize cross-contamination.

Set up all reaction mixtures in an area separate from those used for DNA preparation or PCR product analysis.

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

### Comments and suggestions

c)	HotStarTaq d-Tect Polymerase activation time too long	Check whether PCR was started with an initial incubation step at 95°C for only 10 min.
d)	Too many cycles	Reduce the number of cycles in steps of 3 cycles.
e)	Mg <sup>2+</sup> concentration not optimal	Perform PCR with different final concentrations of $Mg^{2+}$ .
		Add additional Mg $^{2+}$ in 0.25 mM steps using a 25 mM MgCl $_2$ solution.
f)	Primer concentration not optimal or primers degraded	Repeat the PCR with different primer concentrations from 0.1–0.5 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.*
g)	Primer design not optimal	Review primer design. Following bisulfite treatment of DNA, both strands are no longer complementary. Therefore, both primers have to be redesigned to match either the forward or the reverse strand.
		We recommend checking PCR specificity and reliability with EpiTect PCR Control DNA (see Ordering Information, page 17).

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

Product	Contents	Cat. no.
Related products		
EpiTect Bisulfite Kits — for comple and cleanup of DNA for methylat		
EpiTect Bisulfite Kit (48)	48 EpiTect Bisulfite Spin Columns, Reaction Mix, DNA Protect Buffer, Carrier RNA, Buffers	59104
EpiTect 96 Bisulfite Kit (2)	2 x EpiTect Bisulfite 96-well Plates, Reaction Mix, DNA Protect Buffer, Carrier RNA, Buffers	59110
EpiTect Control DNA — for evaluation analysis	ation of PCR primers used	
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 PCR Control DNA Set (100)	Human control DNA set (containing both bisulfite converted methylated and unmethylated DNA and unconverted unmethylated DNA) for 100 control PCRs	59695
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 (100)	REPLI-g Midi DNA Polymerase, EpiTect WBA Reaction Buffer, Nuclease-Free Water for 100 whole bisulfitome amplification reactions	59205

Product	Contents	Cat. no.	
EpiTect MethyLight PCR Kit — for of methylation status	real-time quantification		
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	
QIAamp DNA Micro Kit — for purification of genomic and mitochondrial DNA from small amounts of fresh or frozen blood, tissue, FFPE tissue, and dried blood spots			
QIAamp DNA Micro Kit (50)	For 50 DNA preps: 50 QIAamp MinElute® Columns, Proteinase K, Carrier RNA, Buffers, Collection Tubes (2 ml)	56304	
QIAamp DNA Mini Kit — for purification of genomic, mitochondrial, bacterial, parasite, or viral DNA from a wide variety of clinical samples			
QIAamp DNA Mini Kit (50)	For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51304	
QIAamp DNA Mini Kit (250)	For 250 DNA preps: 250 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51306	

Product	Contents	Cat. no.	
EZ1® DNA Tissue Kit — for automated purification of high-quality DNA from 1–6 tissue samples using EZ1 workstations			
EZ1 DNA Tissue Kit (48)	For 48 DNA preps: 48 Reagent Cartridges (Tissue), Disposable Tip Holders, Disposable Filter-Tips, Sample Tubes (2 ml), Elution Tubes (1.5 ml), Buffer G2, Proteinase K	953034	
EZ1 DNA Tissue Card	Preprogrammed card for BioRobot® EZ1 DNA Tissue Protocol	9015588	
EZ1 Advanced DNA Tissue Card	Preprogrammed card for purification of DNA using the EZ1 Advanced	9018295	
	ation of total cellular DNA from animal		
tissues and cells, yeast, or bacter			
DNeasy Tissue Kit (50)*	For 50 DNA minipreps: 50 DNeasy Spin Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	69504	
DNeasy 96 Tissue Kit (4)*†	For 4 x 96 DNA minipreps: 4 DNeasy 96 Plates, Proteinase K, Buffers, S-Blocks, AirPore Tape Sheets, Collection Microtubes (1.2 ml), Elution Microtubes RS, Caps, 96-well Plate Registers	69581	
QIAamp DNA Blood Kits — for purification of genomic, mitochondrial, or viral DNA from blood and related body fluids			
QIAamp DNA Blood Mini Kit (50)*	For 50 DNA minipreps: 50 QIAamp Mini Spin Columns, QIAGEN Protease, Reagents, Buffers, Collection Tubes (2 ml)	51104	
QIAamp DNA Blood Midi Kit (20)*	For 20 DNA midipreps: 20 QIAamp Midi Spin Columns, QIAGEN Protease, Buffers, Collection Tubes (15 ml)	51183	

<sup>\*</sup> Larger kit sizes available; see  $\underline{www.qiagen.com}$  .

<sup>&</sup>lt;sup>†</sup> Requires use of the QIAGEN 96-Well-Plate Centrifugation System.

Product	Contents	Cat. no.
QIAamp DNA Blood Maxi Kit (10)*	For 10 DNA maxipreps: 10 QIAamp Maxi Spin Columns, QIAGEN Protease, Buffers, Collection Tubes (50 ml)	51192
EZ1 DNA Blood Kits — for automosamples using EZ1 workstations	ated purification of DNA from 1-6 blood	
EZ1 DNA Blood 200 µl Kit (48)	For 48 DNA preps: 48 Reagent Cartridges (Blood 200 µl), Disposable Tip Holders, Disposable Filter-Tips, Sample Tubes (2 ml), Elution Tubes (1.5 ml)	951034
EZ1 DNA Blood 350 µl Kit (48)	For 48 DNA preps: 48 Reagent Cartridges (Blood 350 µl), Disposable Tip Holders, Disposable Filter-Tips, Sample Tubes (2 ml), Elution Tubes (1.5 ml)	951054
EZ1 DNA Blood Card	Preprogrammed card for BioRobot EZ1 DNA Blood 200 µl and 350 µl Protocols	9015585
EZ1 Advanced DNA Blood Card	Preprogrammed card for purification of DNA using the EZ1 Advanced	9018293

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

<sup>\*</sup> Larger kit sizes available; see www.qiagen.com .

### Notes

### Notes

Trademarks: QIAGEN®, QIAamp®, BioRobot®, DNeasy®, EpiTect®, EZ1®, HotStarTaq®, MinElute®, REPLI-g® (QIAGEN Group).

Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

NOTICE TO PURCHASER – LIMITED LICENSE: A license under U.S. Patents 5,786,146, 6,017,704, 6,265,171 and 6,200,756 or their foreign counterparts, owned by The Johns Hopkins University, is required to practice methylation-specific polymerase chain reaction and related processes ("MSP") described in said patents. The purchase price of this product includes limited, nontransferable rights to use only this amount of the product to practice MSP and related processes described in said patents solely for the internal research activities of the purchaser. No right to any other application, including any in vitro diagnostic application, or to perform or offer commercial services of any kind using MSP, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is hereby conveyed or granted by implication or estoppel. Further information on obtaining licenses to practice MSP may be directed to the Licensing Department, OncoMethylome Sciences, 2505 Meridian Parkway, Suite 310, Durham, NC 27713.

Please note that a separate license under certain patents owned by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche Ltd may be required in order to practice the Polymerase Chain Reaction ("PCR") and related processes.

#### **Limited License Agreement**

Use of this product signifies the agreement of any purchaser or user of the EpiTect MSP Kit to the following terms:

- The EpiTect MSP Kit may be used solely in accordance with the EpiTect MSP 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 MSP Handbook
  and additional protocols available at www.giagen.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.

For updated license terms, see www.giagen.com.

© 2008-2014 QIAGEN, all rights reserved.

#### www.qiagen.com

Australia = techservice-au@qiagen.com

Austria = techservice-at@qiagen.com

Belgium = techservice-bnl@qiagen.com

Brazil = suportetecnico.brasil@qiagen.com

Canada = techservice-ca@qiagen.com

China = techservice-cn@qiagen.com

Denmark = techservice-nordic@qiagen.com

Finland = techservice-nordic@qiagen.com

France = techservice-fr@qiagen.com

Germany = techservice-de@qiagen.com

Hong Kong = techservice-hk@qiagen.com

India = techservice-india@qiagen.com

Ireland = techservice-uk@qiagen.com

Italy = techservice-it@qiagen.com

Japan = techservice-jp@qiagen.com

Korea (South) = techservice-kr@qiagen.com

Luxembourg = techservice-bnl@qiagen.com

Mexico = techservice-mx@qiagen.com

The Netherlands = techservice-bnl@qiagen.com

Norway = techservice-nordic@giagen.com

Singapore = techservice-sg@qiagen.com

Sweden = techservice-nordic@qiagen.com

Switzerland = techservice-ch@qiagen.com

UK = techservice-uk@qiagen.com

USA = techservice-us@qiagen.com

