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EpiTect® HRM™ PCR Handbook

For detection and quantification of CpG methylation in bisulfite-treated DNA by high-resolution melting (HRM) analysis

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Kit Contents

EpiTect HRM PCR Kit (100)	**100**
Catalog no.	**59445**
Number of reactions (25 µl)	**100**
2x EpiTect HRM Master Mix , containing: ■ HotStarTaq® *Plus* DNA Polymerase ■ EpiTect HRM PCR Buffer (with EvaGreen® dye) ■ dNTP mix (dATP, dCTP, dGTP, dTTP)	1 x 1.3 ml
RNase-Free Water	1 x 2 ml
Handbook	1

Storage

EpiTect HRM PCR Kit is shipped on dry ice. The kit should be stored immediately upon receipt at –20°C in a constant-temperature freezer and protected from light. 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.

The 2x EpiTect HRM PCR Master Mix can be stored at 2–8°C for up to 2 months without showing any reduction in performance.

Product Use Limitations

EpiTect HRM PCR Kit is intended for molecular biology applications. These products are neither intended for the diagnosis, prevention, or treatment of a disease, nor have they been validated for such use either alone or in combination with other products. Therefore, the performance characteristics of the products for clinical use (i.e., diagnostic, prognostic, therapeutic, or blood banking) are unknown.

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 EpiTect HRM PCR 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 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/support/MSDS.aspx 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

Product Specifications

EpiTect HRM PCR Kit

2x EpiTect HRM PCR Master Mix contains:

HotStarTaq *Plus* DNA Polymerase:	HotStarTaq *Plus* DNA Polymerase is a modified form of a recombinant 94 kDa DNA polymerase, originally isolated from *Thermus aquaticus*, cloned into *E. coli*. (Deoxynucleoside-triphosphate: DNA deoxynucleotidyl-transferase, EC 2.7.7.7). The enzyme is activated by a 5-minute, 95°C incubation step.
EpiTect HRM PCR Buffer:	Novel PCR buffer for highly specific amplification of bisulfite treated DNA followed by high resolution melting analysis.
EvaGreen:	Novel dsDNA binding fluorescent dye for highly efficient and inhibition-free PCR amplification and ideally suited for HRM analysis.
dNTP mix:	Contains dATP, dCTP, dGTP, and dTTP of ultrapure quality.
RNase-free water:	Ultrapure quality, PCR-grade.

Quality Control

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

Introduction

The EpiTect HRM PCR Kit provides a convenient master mix format for detection and quantification of methylated CpGs in bisulfite converted DNA via high-resolution melting analysis (HRM). HRM technology enables rapid characterization of DNA samples based on their melting behavior following PCR amplification. The kit contains the novel dsDNA-binding fluorescent dye, EvaGreen, and combines an optimized HRM buffer and HotStarTaq *Plus* DNA Polymerase to avoid nonspecific amplification products and provide reliable results.

The EpiTect HRM PCR Kit enables simple and rapid screening for methylation differences between samples at various loci.

HRM (High-Resolution Melting)

High-resolution melting (HRM) analysis is an innovative technique that is based on analysis of DNA melting. HRM characterizes DNA samples according to their dissociation behavior as they transition from double-stranded DNA (dsDNA) to single-stranded DNA (ssDNA) with increasing temperature.

Before performing HRM analysis, the target sequence must be amplified to a high-copy number in the presence of the dsDNA binding fluorescent dye, EvaGreen. The dye does not interact with single-stranded DNA (ssDNA) but actively binds to dsDNA and fluoresces brightly when bound. Change in fluorescence can be used to measure the increase in DNA concentration during PCR and then to directly measure thermally-induced DNA melting by HRM.

To perform high-resolution melting analysis, the temperature is increased from a lower to a higher temperature. The fluorescence of EvaGreen is measured continuously as the temperature is increased and is plotted against the temperature. EvaGreen fluoresces as long as it is bound to dsDNA. Due to the amplification procedure before the HRM analysis, fluorescence will be high at the beginning of the HRM analysis. Upon melting of dsDNA, EvaGreen is released and the fluorescence is reduced to a background level.

HRM analysis is an easy and cost-effective alternative to probe-based methylation assays.

2x EpiTect HRM PCR Master Mix

EpiTect HRM PCR Master Mix ensures highly specific amplification, as well as flexible, rapid, and sensitive analysis of the methylation status of CpG dinucleotides in bisulfite converted DNA via high-resolution melting.

The components of the 2x EpiTect HRM PCR Master Mix include HotStarTaq *Plus* DNA Polymerase, EpiTect HRM PCR Buffer, and dNTPs. The optimized master

mix ensures that specific PCR products are amplified with high efficiency for successful HRM analysis.

HotStarTaq *Plus* DNA Polymerase

HotStarTaq *Plus* DNA Polymerase is a modified form of QIAGEN *Taq* DNA Polymerase, and is provided in an inactive state and has no enzymatic activity at ambient temperature. This prevents the formation of misprimed products and primer–dimers during reaction setup and the first denaturation step. Competition for reactants by PCR artifacts is therefore avoided, enabling high PCR specificity and accurate quantification. The enzyme is activated at the start of a reaction by a 5-minute, 95°C incubation step. The hot start enables reactions to be set up rapidly and conveniently at room temperature.

EvaGreen

EvaGreen is a novel fluorescent dye which selectively binds to dsDNA. Upon binding, fluorescence is strongly increased. The spectral properties of EvaGreen are very similar to those of SYBR® Green I. The absorbance maximum is at 500 nm (with DNA bound) and the emission maximum is at 530 nm. This allows easy detection of EvaGreen on channels/filters preset for HRM analysis and SYBR Green detection. In contrast to SYBR Green I, EvaGreen can be used in higher concentrations and shows equal binding affinity for GC-rich and AT-rich regions with no apparent sequence preference. This makes EvaGreen an ideal dye for HRM analyses of all types of PCR products.

Protocol

The EpiTect HRM PCR Kit has been optimized for use with the following real-time cyclers:

LightCycler 480, Rotor-Gene® Q, and Rotor-Gene 6000.

General considerations for CpG methylation analysis by HRM

Always check the real-time PCR instrument manual for details on HRM setup and analysis on your instrument.

Template

- Purified genomic DNA of every origin suitable for PCR with respect to purity and concentration can be used to successfully perform conversion by bisulfite treatment.
- It is recommended to use the same genomic DNA purification procedure for all samples to be converted. This avoids introduction of variations due to differing compositions of elution buffers used in different extraction methods.
- For CpG methylation analysis, the DNA has to be completely converted by bisulfite treatment. To avoid any reduction in performance, we recommend using the EpiTect Bisulfite Kit for complete bisulfite conversion and cleanup of DNA prior to HRM methylation analysis.
- Converted DNA of every origin can be used to successfully perform HRM with the EpiTect HRM PCR Kit.
- **Important:** Use comparable amounts of template genomic DNA for all samples resulting in C_T values below 30 and differing by no more than 3 C_T values.
- DNA samples used for HRM should be normalized in concentration. All DNA samples should be quantified and then adjusted to the same concentration using the same dilution buffer.
- For samples strongly differing in DNA integrity, use appropriate amounts that will result in comparable C_T values. Note that C_T values should always be below 30. All samples analyzed with the same pair of primers should not differ by more than three C_T values.
- Use sufficient PCR cycles so that all samples have reached the plateau phase of PCR to ensure that comparable amounts of PCR product are generated. Note that the amount of DNA affects the melting temperature of the PCR product. Check protocol for details.

Assay design

- Design assays with PCR product lengths of 70–200 bp. Larger products can be analyzed successfully but usually provide lower resolution. This is

because, for example, a single base variation has a greater effect on the melting behavior of a 100 bp amplicon than on a 500 bp amplicon.

Primers

- Design primers allowing specific amplification of the bisulfite converted DNA. 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.
- The melting temperature of primers used for PCR with subsequent HRM analysis should be at least 62°C. The melting temperature of primers can be calculated using the formula below:
  T_m = 2°C x (number of [A+T]) + 4°C x (number of [G+C])
- Whenever possible, design primer pairs with similar T_m values.
- Check the concentration and integrity of primers before starting. Typically, standard primer quality primers are sufficient for HRM. For details, see Appendix C.
- Start with a final primer concentration of 0.75 µM.

HRM protocol

- It is required to initially determine the melting point for each new HRM PCR product. Run HRM analysis to span a temperature range from 65°C–95°C, covering the full range of expected melting points. In future experiments, after determination of the T_m, you may run HRM from 5°C below the lowest T_m of all expected PCR products to 5°C above the highest T_m of all PCR products in your experiment. This may reduce the time needed for HRM analysis.
- **Optional:** Insert a denaturation step at 95°C for 30 seconds and a prehold temperature of 50°C for 30 seconds prior to the high-resolution melt. This will ensure that all products have reassociated and will encourage heteroduplex formation, which may improve results in some instances.

Data analysis

- Check that PCR contains only specific product. Samples showing post-PCR artifacts such as primer–dimers or nonspecific products can make HRM results difficult to interpret. The EpiTect HRM PCR Kit ensures maximum specificity with minimal need for optimization.

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.

- Primers: The EpiTect HRM PCR Kit can be used with standard quality primers that can be purchased from established oligonucleotide manufacturers. Lyophilized primers should be dissolved in TE buffer to provide a stock solution of 100 μM; concentration should be checked by spectrophotometry. Primer solutions should be stored in aliquots at –20°C. Avoid repeated freeze/thaw cycles of primers. Prepare working solutions by dilution in low amounts of TE (e.g. each primer at 10 μM) and store in small aliquots for single use. Primers can also be stored as a ready-to-use mix containing the forward and the reverse primer at equimolar concentrations.
- Nuclease-free (DNase-free) consumables: special care should be taken to avoid nuclease contamination of all reagents and consumables used to set up PCR.
- Optical PCR tubes or plates (use thin-walled PCR tubes or plates recommended by the manufacturer of your real-time cycler).
- **Optional:** Trizma® base and EDTA for preparing TE buffer for storing primers (see Appendix C). Use RNase/DNase-free water and plastic consumables to prepare TE buffer.

Important Notes

No template control (NTC)

All detection experiments should include an NTC, containing all the components of the reaction except for the template. This enables detection of potential contamination.

Positive control

It is useful to include one or more positive controls consisting of bisulfite converted DNA of known methylation status. We recommend using the EpiTect Control DNA Set for this purpose (cat no. 59695).

Protocol: Analysis of CpG Methylation in Bisulfite Converted DNA

This protocol was tested with Rotor-Gene Q, Rotor-Gene 6000, and LightCycler 480.

Important points before starting

- Always start with the cycling conditions specified in this protocol.
- Use the primer concentrations specified in this protocol.
- Optimal instrument and HRM analysis settings are a prerequisite for accurate results. For details, please refer to the manual provided with your HRM real-time PCR instrument procedure.

Procedure

1. **Thaw the EpiTect HRM PCR Master Mix, primer solutions, RNase-free water, template DNAs, and the control DNAs.**

   It is important to mix the solutions completely before use to avoid localized concentrations of salt.

2. **Prepare a reaction mix according to Table 1 (page 14).**

   It is recommended to prepare a volume of reaction mix 10% greater than that required for the total number of reactions to be performed.

   Typically, reaction setup can be done at room temperature (15–25°C). However, it is recommended to keep the individual reagents, samples, and controls on ice.

3. **Mix the reaction mix thoroughly, and dispense appropriate volumes into PCR tubes or the wells of a PCR plate.**

4. **Add equal amounts and volume of template DNA (5–10 ng bisulfite converted DNA) to the individual PCR tubes or wells and mix thoroughly.**

   Add sufficient DNA that all samples show C_T values below 30. Samples should not differ by more than three C_T values.

Table 1. Reaction composition using EpiTect HRM PCR Master Mix, 2x

Component	Volume per 25 µl reaction*	Volume per 10 µl reaction*	Final concentration
Reaction mix			
2x EpiTect HRM PCR Master Mix	12.5 µl	5 µl	1x
10 µM (each) primer mix†	1.9 µl	0.75 µl	0.75 µM forward primer 0.75 µM reverse primer
RNase-free water	Variable	Variable	–
Template DNA‡ (added at step 4)	Variable	Variable	5–10 ng/reaction §
Total volume per reaction	**25 µl***	**10 µl***	–

* If your real-time cycler requires a final reaction volume other than 25 µl or 10 µl, adjust the amount of master mix and all other reaction components accordingly. † A 10 µM primer mix consists of 10 µM forward primer and 10 µM reverse primer. ‡ It is recommended to use concentration normalized DNA samples. § Maximum template amount is ≤50 ng.

5. **Program the real-time cycler according to Tables 2 or 3 (pages 15–16).**

   Note: Check the real-time cycler's user manual for correct instrument setup.

6. **Place the PCR tubes or plate in the real-time cycler, and start the PCR cycling program, followed by HRM analysis.**

7. **Perform data analysis.**

   Before performing data analysis for real-time PCR and HRM, specify the analysis settings. See real-time PCR instrument manual for details.

Table 2. Optimized cycling protocol for HRM analysis on the Rotor-Gene Q and Rotor-Gene 6000

			Additional comments
Initial PCR activation step	**5 min**	**95°C**	HotStarTaq *Plus* DNA Polymerase is activated by this heating step.
3-step cycling:			**Important: Optimal performance is only assured using these cycling conditions**
Denaturation	**10 s**	**95°C**	
Annealing	**30 s**	**55°C**	
Extension	**10 s**	**72°C**	Fluorescence data acquisition on the "Green" channel. Suitable for PCR products up to 150 bp. For longer PCR products, use 8 s extension time per 100 bp of PCR product length.
Number of cycles	45 40		5–10 ng template DNA 11–50 ng template DNA
HRM analysis for: **Rotor-Gene Q**	2 s	65–95° C 0.1 °C increments	Fluorescence data acquisition on the "HRM" channel, for details see page 9.

* It is required to initially determine the melting point for each new HRM PCR product. Run HRM analysis to span a temperature range from 65°C–95°C, covering the full range of expected melting points. In future experiments, after determination of the *T*m, you may run HRM from 5°C below the lowest *T*m of all expected PCR products to 5°C above the highest *T*m of all PCR products in your experiment. This may reduce the time needed for HRM analysis.

Table 3. Optimized cycling protocol for HRM analysis on the LightCycler 480

			Additional comments
			Choose detection format: SYBR Green I/HRM Dye
Initial PCR activation step	**5 min**	**95°C**	HotStarTaq *Plus* DNA Polymerase is activated by this heating step
3-step cycling:			**Important: Optimal performance is only assured using these cycling conditions.** **Maximal ramp rates for the three cycling steps should be used.**
Denaturation	**10 s**	**95°C***	
Annealing	**30 s**	**55°C***	
Extension	**10 s**	**72°C***	Activate "single" fluorescence data acquisition. Suitable for PCR products up to 150 bp. For longer PCR products, use 8 s extension time per 100 bp of PCR product length.
Number of cycles	45		5–10 ng template DNA
	40		11–50 ng template DNA
HRM			Analysis mode: Melting curve
	1s	65°C†	
		95° C†	Continuous fluorescence data acquisition. Ramp rate: 0.02 °C/s 25 acquisitions per second
Cooling	1s	40°C	Cooling samples after HRM

* Use max ramp rates for heating and cooling. † It is required to initially determine the melting point for each new HRM PCR product. Run HRM analysis to span a temperature range from 65°C–95°C, covering the full range of expected melting points. In future experiments, after determination of the *T*m, you may run HRM from 5°C below the lowest *T*m of all expected PCR products to 5°C above the highest *T*m of all PCR products in your experiment. This may reduce the time required for HRM analysis.

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 signal (PCR or HRM) or signal detected late in PCR	
a) Wrong assay design	Verify assay design. Design primers allowing specific amplification of the bisulfite converted DNA.
b) Extension time too short	Always use the extension times specified in the protocol (10 s for up to 150 bp, for longer amplicons, use 8 s/100 bp).
c) Wrong cycling conditions	Always start with the optimized cycling conditions specified in the protocol.
d) HotStarTaq *Plus* DNA Polymerase not activated	Ensure that the cycling program includes the HotStarTaq *Plus* DNA Polymerase activation step (5 min at 95°C) as described in the protocol.
e) Pipetting error or missing reagent	Check the concentrations and storage conditions of the reagents, including primers and template nucleic acid. See Appendix C (page 21) for details on evaluating the concentration of primers. Repeat the assay.
f) Wrong or no detection step in real-time analysis	For real-time analysis, ensure that fluorescence detection takes place during the 72°C extension step.
g) Poor PCR efficiency	Use the primer concentrations given in the protocol. See Appendix C (page 21) for details on determining the concentration of primers Avoid repeated freezing and thawing of primers. Prepare small aliquots and only thaw a few times.

	Comments and suggestions
h) PCR product too long	For optimal results, PCR products should be between 70 and 200 bp. PCR products should not exceed 300 bp.
i) Annealing temperature too high	Decrease annealing temperature in 3°C steps.
j) Mg^{2+} concentration not optimal	Mg^{2+} concentration is optimal for most of the targets. For a few targets, an increase up to 3 mM Mg^{2+} may be helpful. Perform the titration in 0.3 mM steps
k) Insufficient amount of starting template	Increase the amount of template up to 50 ng, if possible. Ensure that sufficient copies of the target nucleic acids are present in your sample.
l) Insufficient number of cycles	Increase the number of cycles.
m) No detection activated	Check that fluorescence detection was activated in the cycling program.
n) Wrong detection step	Ensure that fluorescence detection takes place during the extension step of the cycling program.
o) Problems with bisulfite conversion reaction	Check the concentration, storage conditions, and quality of the bisulfite converted and control DNA. If necessary, make new serial dilutions of the template DNA from the stock solutions. Repeat the assay using the new dilutions. Efficient bisulfite conversion and removal of PCR inhibitors is essential for optimal results. Perform bisulfite treatment of nucleic acids from your sample using an appropriate bisulfite conversion method, e.g., using EpiTect Bisulfite Kits.
p) Insufficient or degraded template DNA	Check if template amount and PCR cycle number were used as specified in the protocol (Tables 1, 2, and 3). Increase the amount of template if possible. Use 40 cycles. Be sure to use 5–10 ng of bisulfite converted DNA. Use the EpiTect Control DNA Set to test PCR results and primer reliability.

	Comments and suggestions
q) Problems with starting template DNA for bisulfite conversion reaction	Check the concentration, storage conditions, and quality of the DNA used for bisulfite conversion. If necessary, make new serial dilutions of the template DNA from the stock solutions. Repeat bisulfite conversion reaction using the new dilutions. Repeat the assay using the newly converted DNA. Ensure that all reagents, buffers and solutions used for isolating and dilution of DNA for bisulfite conversion reaction is free of nucleases.
Increased fluorescence or C_T value for "No Template" control	
a) Contamination of reagents	Discard all the components of the HRM PCR assay (e.g., master mix, primers). Repeat the assay using new components and decontaminated pipettes and consumables.
b) Contamination of real-time cycler	Decontaminate the real-time cycler according to the manufacturer's instructions.
Variability in signal (C_T and/or Rn in HRM) between replicates or samples	
a) Problem with template DNA	Recheck the DNA concentrations of the samples. Ensure that comparable amounts of DNA are used in all samples. Use a different HRM assay for methylation analysis to check the integrity of the genomic DNA in all samples.
b) Bubbles in the wells	Spin down plates to remove air bubbles and remove any liquid form the plate cover. Repeat the post-PCR plate read. If your instrument does not allow repeated post-PCR plate read, prepare a new reaction plate, making sure the plate is centrifuged before performing PCR.
c) Reaction components improperly mixed	Follow mixing procedures in the protocol.
d) Contamination of real-time cycler	Decontaminate the real-time cycler according to the manufacturer's instructions

	Comments and suggestions
e) Real-time cycler no longer calibrated	Recalibrate the real-time cycler according to the manufacturer's instructions.

Appendix A: HRM Instrument Setup and Data Analysis

Please refer to your real time cycler user manual for correct instrument setup and data analysis.

If using the Rotor-Gene Q, refer to section 11 (HRM data analysis) of the user manual.

Appendix B: Starting Template

Sample degradation should be avoided during purification and storage. Avoid excessive amounts of inhibitors from ethanol carryover. To improve HRM results, we recommended keeping the amount of template used consistent between samples. Spectrophotometric analysis for determining DNA concentration and purity is recommended. We recommend QIAGEN kits for sample preparation and EpiTect Bisulfite Kits for bisulfite conversion and cleanup of sample DNA. **Note**: At 260 nm one absorbance unit is equal to 50 µg/ml DNA. Pure DNA will provide a 260 nm to 280 nm ratio of 1.8.

Appendix C: Handling of Primers

Handling and storing primers

Guidelines for handling and storing primers are given below. For optimal results, we recommend only combining primers of comparable quality.

Storage buffer

Lyophilized primers should be dissolved in a small volume of low-salt buffer to give a concentrated stock solution (e.g., 100 µM). We recommend using TE buffer (10 mM Tris·Cl, 1 mM EDTA, pH 8.0) for standard primers labeled with most fluorescent dyes.

Storage

Primers should be stored in sterile, nuclease-free TE buffer in small aliquots at –20°C. Standard primers are stable under these conditions for at least 1 year.

Dissolving primers

Before opening a tube containing lyophilized primer, centrifuge the tube briefly to collect all material at the bottom of the tube. To dissolve the primer, add the required volume of sterile, nuclease-free TE buffer, mix, and leave for

20 minutes to allow the primer to completely dissolve. Mix again and determine the concentration by spectrophotometry as described below.

We do not recommend dissolving primers in water. They are less stable in water than in TE buffer and some may not dissolve easily in water.

Concentration

Spectrophotometric conversion for primers:

$1\ A_{260}$ unit = 20–30 μg/ml

To check primer concentration, the molar extinction coefficient (ε_{260}) can be used:

$A_{260} = \varepsilon_{260}$ x molar concentration of primer or probe

If the ε_{260} value is not given on the data sheet supplied with the primers or probes, it can be calculated from the primer sequence using the following formula:

$\varepsilon_{260} = 0.89 \times [(A \times 15{,}480) + (C \times 7340) + (G \times 11{,}760) + (T \times 8850)]$

Example

Concentration of diluted primer: 1 μM = 1×10^{-6} M

Primer length: 24 nucleotides with 6 each of A, C, G, and T bases

Calculation of expected A_{260}: $0.89 \times [(6 \times 15{,}480) + (6 \times 7340) + (6 \times 11{,}760) + (6 \times 8850)] \times (1 \times 10^{-6}) = 0.232$

The measured A_{260} should be within ±30% of the theoretical value. If the measured A_{260} is very different to the theoretical value, we recommend recalculating the concentration of the primers, or having the primers resynthesized.

Primer quality

The quality of 18–30mers can be checked on a 15% denaturing polyacrylamide gel;* a single band should be seen. Please call one of the QIAGEN Technical Service Departments or local distributors for a protocol (see back cover) or visit www.qiagen.com.

* 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.

Ordering Information

Product	Contents	Cat. no.
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 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
QIAamp DNA Kits — for genomic, mitochondrial, bacterial, parasite, or viral DNA		
QIAamp DNA Mini Kit (50)*	For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51304
DNeasy Blood & Tissue Kits — for purification of total DNA from animal blood and tissues, and from cells, yeast, bacteria, or viruses		
DNeasy Blood & Tissue Kit (50)*	50 DNeasy Mini Spin Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	69504

* Larger kit sizes available; see www.qiagen.com.

All kits are intended for molecular biology applications. These products are neither intended for the diagnosis, prevention, or treatment of a disease, nor have they been validated for such use either alone or in combination with other products.

Notes

Notes

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