

# EpiTect<sup>®</sup> Control DNA (human), unmethylated and bisulfite converted (100)

## For 100 control reactions for methylation analysis

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

The control DNA is shipped at room temperature and should be stored at  $-20^{\circ}\text{C}$  immediately upon receipt. When stored under these conditions and handled correctly, this product can be kept for at least 6 months without showing any reduction in performance.

The unmethylated and bisulfite converted DNA is stored in EB Buffer (10 mM Tris·Cl) in a ready-to-use 10 ng/ $\mu\text{l}$  solution.

Bisulfite conversion of control DNA was achieved using the EpiTect Bisulfite Kit.

## Important handling notes

- 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.
- We strongly recommend EpiTect Bisulfite Kits for bisulfite conversion reactions.

## Procedure

Use 1  $\mu\text{l}$  (10 ng) of each control DNA for every PCR reaction.

We recommend using the EpiTect MSP Kit or EpiTect MethyLight PCR Kit for highly specific and reliable methylation-specific PCR results (see Table 1 and 2 for reaction setup).



**Table 1. Reaction Setup Using EpiTect MSP Kit**

Component	Volume/reaction	Final concentration
<b>EpiTect Master Mix, 2x</b>	25 $\mu$ l	1x
<b>Diluted primer mix</b>		
Primer A*	variable	0.3–0.4 $\mu$ M
Primer B*	variable	0.3–0.4 $\mu$ M
RNase-free water	variable	–
<b>Template DNA</b>	1 $\mu$ l	10 ng
<b>Total volume</b>	<b>50 <math>\mu</math>l</b>	

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

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

**Table 2. Reaction Setup Using EpiTect MethyLight PCR Kit**

Component	Volume/50 $\mu$ l reaction	Final concentration
<b>Reaction mix</b>		
EpiTect MethyLight Master Mix, 2x	25 $\mu$ l	1x
<b>10x primer–probe mix</b>	5 $\mu$ l	0.4 $\mu$ M forward primer* 0.4 $\mu$ M reverse primer* 0.2 $\mu$ M probe (0.2 $\mu$ M of each probe when using quantitative MethyLight Assays)
RNase-free water	variable	–
<b>Template DNA</b>	1 $\mu$ l	$\leq$ 100 ng/reaction
(added at step 4)		
<b>Total volume</b>	<b>50 <math>\mu</math>l</b>	

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

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

**Table 3. Cycling conditions for methylation PCR analysis using TaqMan probes**

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

## Ordering Information

Product	Contents	Cat. no.
<b>EpiTect Control DNA — for evaluation of PCR primers used for methylation analysis</b>		
EpiTect Control DNA, methylated (100)	Methylated and bisulfite converted human control DNA for 100 control PCRs	59655
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
<b>Related products</b>		
<b>EpiTect Bisulfite Kit — for complete bisulfite conversion and cleanup of DNA for methylation analysis</b>		
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

# Ordering Information

Product	Contents	Cat. no.
<b>EpiTect MethyLight PCR Kit — for optimal probe based real-time PCR (for quantification of methylated or unmethylated CpG sites)</b>		
EpiTect MethyLight PCR Kit (200)*	Master Mix for methylation-specific real-time PCR analysis, 200 x 50 µl	59436
EpiTect MethyLight PCR + ROX Vial Kit (200)*	Master Mix without ROX for methylation-specific real-time PCR analysis, 200 x 50 µl	59496
<b>EpiTect MSP Kit — for improved methylation-specific end-point PCR analysis</b>		
EpiTect MSP Kit (100)*	EpiTect MSP Master Mix for 100 x 50 µl reactions	59305
<b>EpiTect Whole Bisulfite Kit — for amplification of bisulfite converted DNA</b>		
EpiTect Whole Bisulfite Kit (25)*	REPLI-g Midi DNA Polymerase, EpiTect WBA Reaction Buffer, nuclease-free water for 25 whole bisulfite amplification reactions	59203

\* Larger kit sizes/formats available; see [www.qiagen.com](http://www.qiagen.com).

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.

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Use of methylation specific PCR (MSP) is covered by US patents 5,786,146, 6,017,704, 6,200,756 & 6,265,171 and corresponding foreign patents and applications. No license under these patents to use the MSP process is conveyed to the purchaser by purchasing this product

The PCR process is covered by the foreign counterparts of U.S. Patents Nos. 4,683,202 and 4,683,195 owned by F. Hoffmann-La Roche Ltd.

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