EpiTect® Control DNA (human), unmethylated (10 µg)

For 1000 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 or unmethylated DNA**.** With this DNA, you can if check the primer is specific for genomic DNA. In addition, this DNA can be used to check the conversion efficiency of bisulfite conversion reactions.

The control DNA is shipped at room temperature and should be stored at –20°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 control DNA is stored in EB Buffer (10 mM Tris·Cl) in a ready-to-use 50 ng/µl solution.

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

For PCR analysis, we recommend the use of 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).

Use 20 µl (1000 ng) of DNA for each bisulfite conversion reaction.

We recommend using the EpiTect Bisulfite Kit or the EpiTect 96 Bisulfite Kit to ensure high conversion efficiencies.

In the EpiTect Bisulfite Kit protocols, the converted DNA is eluted in 40 – 50 µl. Use 1 µl of bisulfite converted DNA for every PCR reaction.

Table 1. Reaction Setup Using EpiTect MSP Kit

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
RNase-free water	variable	–
Template DNA	1 µl	10 ng
Total volume	**50 µl**	

* 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.

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 µl reaction	Final concentration
Reaction mix		
EpiTect MethyLight Master Mix, 2x	25 µl	1x
10x primer–probe mix	5 µl	0.4 µM forward primer* 0.4 µM reverse primer* 0.2 µM probe (0.2 µM of each probe when using quantitative MethyLight Assays)
RNase-free water	variable	–
Template DNA (added at step 4)	1 µl	≤100 ng/reaction
Total volume	**50 µl**	

* 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.

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
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

Ordering Information

Product	Contents	Cat. no.
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 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
Related products		
EpiTect Bisulfite Kit — 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)	2 x EpiTect Bisulfite 96-well Plates, Reaction Mix, DNA Protect Buffer, Carrier RNA, Buffers	59110

Ordering Information

Product	Contents	Cat. no.
EpiTect MethyLight PCR Kit — for optimal probe based real-time PCR (for quantification of methylated or unmethylated CpG sites)		
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
EpiTect MSP Kit — for improved methylation-specific end-point PCR analysis		
EpiTect MSP Kit (100)*	EpiTect MSP Master Mix for 100 x 50 µl reactions	59305
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

* Larger kit sizes/formats available; see 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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