

EpiTect™ MethyLight Assay: Hs_PITX2

TaqMan® probe based real-time quantification of methylated or unmethylated CpG sites

EpiTect MethyLight Assays enable discrimination between unmethylated and methylated sites in the same sample. The FAM labeled TaqMan probe always detects the methylated site whereas the VIC® labeled TaqMan probe always detects the unmethylated site.

Each MethyLight Assay provides 500 µl of a ten-fold primer–probe mix in a ready-to-use solution.

EpiTect MethyLight Assays are developed, validated and optimized for use with EpiTect MethyLight PCR Kits (see Ordering Information).

For each EpiTect MethyLight reaction, a volume of 5 µl of the premixed 10x EpiTect MethyLight Assay is required in a final real-time PCR volume of 50 µl.

Important handling notes

- Use of EpiTect MethyLight PCR Kits is strongly recommended.
- EpiTect MethyLight Assays are optimized for a starting amount of 10 ng bisulfite converted human genomic DNA.
- EpiTect MethyLight Assays consist of TaqMan probes, therefore fluorescence must be detected during the combined annealing/ extension step.
- Optimal performance of EpiTect MethyLight Assays is only assured using the cycling conditions listed in Table 2. When using other than QIAGEN real-time Probe PCR kits (with HotStarTaq® Plus DNA Polymerase), please follow the recommended initial enzyme activation step in the handbook.
- EpiTect MethyLight Assays have been optimized for a final reaction volume of 50 µl per tube. If other volumes are used, adjust the amount of 10x EpiTect MethyLight Assay accordingly. An example for correct set-up is listed in Table 1.
- Prevent repeated freeze and thaw cycles. To maintain optimal performance, these should be limited to a maximum of ten cycles. Alternatively, aliquot the primer–probe mix.



Specifications

Official gene name	Paired-like homedomain 2
Official gene symbol	PITX2
Species	Human (Homo sapiens)
Entrez Gene ID	5308
RefSeq ID	NT_016354.18
Detected CpG in RefSeq	3 CpG in 36106573 - 36106600
Amplicon length	144, chromosome 4
Dye label unmethylated	VIC
Dye label methylated	FAM
Assay type	Functionally validated with EpiTect MethyLight PCR Kit

Table 1. Preparing a reaction mix for quantitative methylation PCR analysis using EpiTect MethyLight Assays

Component	Volume per 50 μl reaction	Final concentration
Reaction mix		
2x EpiTect MethyLight Master Mix	25 μ l	1x
MethyLight Primer Probe Mix 10x	5 μ l	1x
RNase-free water	Variable	–
Template DNA (added at step 4)	Variable	\leq 100 ng/reaction
Total volume per reaction	50 μl	–

Table 2. Cycling conditions for methylation PCR analysis using TaqMan probes

Step	Time	Temperature	Additional comments
Initial PCR activation step	5 min	95°C	HotStarTaq <i>Plus</i> 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.
Hs_PITX2*	10-fold primer TaqMan probe mix, for real-time MethyLight PCR	59930
EpiTect MethyLight PCR Kits — 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 Control DNA — for evaluation of PCR primers used for methylation analysis		
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

*Other assays available; see www.qiagen.com .

† Other kit sizes/formats available; see www.qiagen.com .

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 Whole Bisulfite Kits — for amplification of bisulfite converted DNA		
EpiTect Whole Bisulfite Kit (25) [†]	REPLI-g Midi DNA Polymerase, EpiTect WBA Reaction Buffer, nuclease-free water	59203

[†] Other kit sizes/formats available; see www.qiagen.com.

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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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