MethyLight qPCR probe Set

Cat. No. : RK21242

Product components

Components	Component number	Size-1 100 RXN(20 μL/RXN)	Size-2 500 RXN(20 μL/RXN)
MethyLight HS Taq Polymerase	RM21231	100 μL	500 μL
MethyLight 5X qPCR Reaction Buffer (No dNTPs and Mg2+)	RM21233	400 μL	1mL*2
dNTPs (10 mM each)	RM20120	50 μL	250 μL
MgCl2 (50 mM)	RM20144	1.25 mL	1.25 mL

Product Description

MethyLight qPCR Probe Set is designed for probe-based qPCR reactions. It is a powerful tool for MSP analysis of methylated DNA and multi-target detection in epigenetic research. Through buffer optimization, it can be used for multiple MSP (methylation specific PCR) analysis on ssDNA containing uracil through DNA methylation by bisulfite or enzyme conversion. MethyLight qPCR Probe Set containing antibody-mediated hot start DNA polymerase, dNTPs, MgCl2, KCI and other stabilizers, improves molecular recognition ability and sensitively identifies methylated and non-methylated sequences. It helps user efficiently obtain accurate quantification and good repeatability.

This product contains antibody-mediated MethyLight HS Taq Polymerase, MethyLight 5X qPCR Reaction Buffer (No dNTPs and Mg2+), dNTPs, and MgCl2 required for MethyLight qPCR reactions except primers, probes and templates.

Storage

-20℃

Compatible Instruments

Reference Dye	Instruments
No ROX	Bio-Rad iCyclers®, Roche Light Cyclers®, QIAGEN/Corbett®; ABI 7000/7300/7700/7900, ABI StepOne™/StepOnePlus™, ABI 7500/ViiA7™, QuantStudio™, Stratagene Real-time PCR Systems, Rotor-gene ™3000, etc.

Material Required

1. EP tubes, qPCR tubes, and aerosol-resistant pipettes and tips
2. qPCR primers and probes, DNA or cDNA templates
3. Tube or qPCR 96-well plate and sealing membrane (adhesive film).

Precautions

1. Fully thaw MethyLight 4X qPCR Reaction Buffer before use.
2. Mix gently before use and avoid air bubbles. Spin briefly to collect all the contents at the tube bottom. After use, return it to -20℃ immediately.
3. This product contains polymerase. Please put it on ice when using it. After first thaw, the master mix is stable at 4 ℃ for 1 week. Avoid repeated freezing and thawing as much as possible.
4. If applicable, use aerosol-resistant pipette tips to minimize contamination.
5. High quality DNA templates are recommended for optimal .

Operation Description

Preparation

1. A final primer concentration of 0.2 μM is recommended for most reactions. However, to optimize individual reaction, a primer titration from 0.1 μM to 1.0 μM can be performed.
2. The length of amplified PCR products should ideally be in the range of 70 - 200 bp.
3. Prepare a serial dilution of the template to access standard curve and test primer efficiency.
4. Use 1 pg~50 ng of DNA template in a 20 μL reaction. Always include a no template control (NTC) reaction.
5. Triplicates are recommended as technical replicates in real-time PCR reactions.

Recommended Reaction

Components	20 μL
MethyLight 5X qPCR Reaction Buffer (No dNTPs and Mg2+)	4 μL
MethyLight HS Taq Polymerase	1 μL
Forward Primer (10 μM)	0.4 μL
Reverse Primer (10 μM)	0.4 μL
dNTPs (10 mM each)	0.5 μL
MgCl2 (50 mM)	1.5 μL
Probe (5 μM)	0.4 μL
ssDNA Template	10 pg~100 ng
ddH_2O	to 20 μL

Note: (1) If the reaction system needs to be changed to 25 μL, the corresponding volume of all reaction solutions in the above list should be increased by 1.25 times.

(2) Calculate the amount of mix need, generally a 10% extra amount is suggested.

(3) Dispense solution in sterile PCR or EP tubes in case of any contamination.

(4) Add all components listed in above table, agitate the tubes gently to mix thoroughly (avoid bubbles) and centrifuge it.

(5) Dispense the reaction solution into qPCR plates and seal the plates with optical membrane.

(6) 2500 rpm centrifuge the qPCR plates to collect all solution.

Recommended PCR Program

Step	Temp	Time	Cycles
Pre-denatura tion	95 ℃	5-10 min	1
Denaturation	95 ℃	15 s	
Annealing	55 ℃	30 s	40~50

Note: 1.To ensure signal acquisition after extension, please adjust the extension temperature according to the Tm value of the primer and probe.

2: In general, It is recommended that the shortest pre-denaturation time should not be shorter than 5 min, and the longest should not exceed 20 min; The shortest denaturation time in the cycle reaction is not less than 1s, and the longest is not more than 15s;The shortest extension time in the cycle reaction is not less than 10s, and the longest can be adjusted according to the needs of primers, probes and signal acquisition.

Data Analysis

1. A standard curve is a linear regression analysis on the data plotted as the Ct values versus the log sample input concentration. If the standard curve correlation coefficient (R2) is >0.98, the template concentration data points are within the linear range of the assay. When the slope of a standard curve is between -3 and -3.5, the PCR amplification efficiency (E) is

between 90 and 120%.

2. Ideally, the standard deviations (STD) of theCtvalues between replicates should be <0.2cycle.For most cases, the STDs of Ct values<0.5cycleare acceptable.

3. The Ct value of a valid amplification should be less than the value of the NTC curve.

Trouble shooting

1) Unusual Amplification Curves

a. Amplification Curve Not Smooth: Too low amplification signal, increase the template input and make sure the qPCR Mix is stored properly.

b. Inconsistent Amplification Curve: Bubbles causes abnormal qPCR results, centrifuge the plate prior to running it.

c. Abnormal Amplification Curves: the default baseline value of machine is set to be from 3 to 15, the baseline setting can be changed according actual amplification conditions. Besides, the degradation of template may affect the curve.

2) No Amplification Curves after Reaction

a. Not Enough PCR Cycles: the PCR cycle number is usually set to be 40. It should be noted a higher cycle number may increase the background signal.

b. Primer Degradation: Use electrophoresis to confirm the integrity of primers.

c. Confirm the Signal Collection Step: the signal collection step are usually set to be after the annealing-extending step for two-step qPCR and after extending step for Three-step qCPR.

d. Template Input Too Low: Increase template concentration or add extra repetition.

e. Template Degradation: Use freshly prepared template (Use electrophoresis to confirm integrity of template).

3) Ct Value Too Late

a. Low Amplification Efficiency: Optimize reaction condition or change primer.

b. Template Input Too Low: Increase template concentration or add extra repeat.

c. Template Degradation: Use freshly prepared template (Use electrophoresis to confirm Integrity of template).

d. Too Long PCR Products: The length of amplification products is usually in the range of 70 bp-200 bp.

e. PCR Inhabitation Reagent: use new template or dilute the template.

4) NTC Shows Amplification

a. Contamination: Use sterile water to conduct experiment and the all operation is suggested to be done in clean room to avoid aerosol contamination.

5) Inconsistent Results

a. Inconsistent Sample Added: Use proper pipetting techniques.

b. Inconsistent Temperature in qPCR Machine: ensure periodic machine calibration.

c. Template Concentration Too Low: the lower template input, the poorer qPCR result is. Increase the template concentration.

d. Inconsistent Threshold Set: when comparing the qPCR results in different plates, make sure the threshold value of each experiments is same.