Taq-HS Probe qPCR Premix (None/Low/High ROX)

REF: EG20118/ EG20119/ EG20120-M

Storage Condition

Store at -30~-15℃ and protect from light. Transport at ≤0℃ .

Components

Component	Amount
Taq-HS Probe qPCR Premix (None ROX)	5×1 ml
Taq-HS Probe qPCR Premix (Low ROX)	5×1 ml
Taq-HS Probe qPCR Premix (High ROX)	5×1 ml

Description

Taq-HS Probe qPCR Premix is a 2× pre-mixed solution developed for real-time quantitative PCR based on the unique "double-block" hot-start Taq DNA polymerase. This product contains all the fluorescent quantitative PCR components except for primers and sample DNA, reducing operational steps, shortening the time, and lowering the risk of contamination. It is ideal for TaqMan probe-based detection using cDNA or DNA as the template.

Due to design variations in some brands and models of fluorescent quantitative PCR instruments, there may be slight differences in the fluorescence signals between PCR wells. Therefore, the addition of a reference dye is necessary for calibration. This product is provided with ROX Reference Dye at different concentrations, please select the appropriate one according to the specific instrument model.

Protocol

1. Equipment selection

Name	Equipment selection
EG20118S (None Rox)	Bio-Rad CFX; Roche LightCycler™; Eppendorf Mastercycler® ep realplex; Qiagen/Corbett Rotor-Gene®; Takara Thermal Cycler Dice; analytikjena qTOWER
EG20119S (Low Rox)	ABI 7500/7500 Fast，ABI ViiA 7™; ABI QuantStudio™; Stratagene Mx3000P®/3005P™/4000™
EG20120S (High Rox)	ABI 7000/7300/7700/7900 HT/7900 HT Fast， StepOne™，StepOne Plus™

2. Notes

① Avoid strong light exposure during usage and storage to protect the pre-mixed dye.

② Mix gentle and thoroughly by turning the tube up and down before use. Do not vortex to generate excessive bubbles.

3. Prepare the following mixture in a qPCR tube

Reagent	Amount	Final concentration
Taq-HS Probe qPCR Premix	10 μl	1×
Forward primer (10 μM)[a]	0.4 μl	0.2 μM
Reverse primer (10 μM)[a]	0.4 μl	0.2 μM
TaqMan probe (5 μM)[b]	1 μl	0.25 μM
DNA template[c]	X μl	10~200 ng/20 μl
Nuclease-Free Water	To 20 μl	

a. Commonly used primer concentration: 0.2 μM final. Adjust between 0.1~1 μM. The primer length should be set at 18~25 bp, with a GC content of 40%~60%. The optimal efficiency for amplifying the target fragment is generally 80~200 bp. When designing, it's important to avoid complex structures such as hairpins, dimers, and to span intron regions whenever possible.

b. Commonly used probe concentration: 0.25 μM final. Adjust between 0.1~1 μM.

c. Suggested template volume: 1~2 μl. Do not exceed 10% of the total volume if the template is undiluted cDNA. As the copy number of target gene in different DNA templates varies, gradient dilution can be performed if necessary to determine the optimal amount of DNA template.

4. qPCR program (Adjust according to the instrument used)

2-step PCR

Step	Temperature	Time
Pre-denaturation	95℃	5 min
Denaturation	95℃	10 s
Annealing & Extension	60℃	30 s

40 Cycles

Notice

1. Before use, please mix it upside down gently to avoid foaming, and centrifuge it briefly before use.

2. Avoid repeated freezing and thawing of Mix, and try to use it up within 3 months after opening.

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FAQ & Troubleshooting

Problem	Possible Reason	Solution
Disordered or missing amplification curves	Incorrect instrument settings	Adjust settings according to the instrument manual
	Improper primer or template concentration	Adjust primer and template concentrations
	Inappropriate PCR reaction conditions	Reduce annealing temperature, extend extension time, etc. For target fragments with high GC content, consider extending the denaturation time appropriately
	Primers or templates with complex secondary structures	Optimize primers
	Poor sample purity	Purify the sample
Poor reproducibility of quantitative values	The instrument settings are incorrect	Adjust settings according to the instrument manual
	Poor sample purity	Purify the sample
	Improper primer concentration	Try increasing the primer concentration appropriately
	Inappropriate PCR reaction conditions	Try reducing the annealing temperature, extending the extension time, etc
	Inappropriate primer design	Redesign primers, Reduce the complex secondary structure of the target fragment
	Experimental operational errors	Strictly follow the operating procedures to ensure accurate volumes of each component in the reaction system
Signal from NTC	Contamination has occurred	Change water, primers, pipette tips, and PCR tubes one by one to find and eliminate the contaminant source. Open a fresh tube of Mix if necessary.

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