Entrans qPCR Probe Set V2

Cat. No. : RK21211

Product components

Components	Component number	Size-1 100 RXN (20 μL/RXN)	Size-2 500 RXN (20 μL/RXN)
Entrans Taq II DNA Polymerase (5, 000 U/mL)	RM21208	20 μL	100 μL
4X qPCR Probe Buffer II (No dNTPs and Mg2+)	RM21211	500 μL	1.25 mL X 2
50X ROX Dye I	RM21465	40 μL	200 μL
50X ROX Dye II	RM21466	40 μL	200 μL
dNTPs (10 mM each)	RM20120	30 μL	125 μL
MgCl2 (50 mM)	RM20144	1.25 mL	1.25 mL

Product Description

Entrans qPCR Probe Set V2 is specialized reagent for qPCR reaction based on probe method. This product uses chemical and antibody double modified hot start Taq DNA polymerase for amplification, which greatly improves the specificity of the product while ensuring the amplification efficiency, and has the characteristics of accurate quantification, good repeatability and wide credibility range. By optimizing the buffer system, the product can be applied for multiple fluorescence channel quantitative tests and is suitable for multiple species, providing a powerful tool for interdisciplinary experimental needs.

This reagent kit provide separate dNTPs and Mg2+ in the reaction buffer, making it convenient for users to optimize the system.

Storage

Upon receipt, store all components at -20℃.

Compatible Instruments

Reference Dye	Instruments
No ROX	Bio-Rad iCyclers/ CFX96/ CFX 384, Roche Light Cyclers® QIAGEN/Corbett Systems
ROX Reference Dye I	Applied Biosystems 7000/7300/7700/7900, Applied Biosystems Step One Plus
ROX Reference Dye II	Applied Biosystems 7500/ViiA7™, QuantStudio™ Stratagene Real-time PCR Systems, Rotor-gene™ 3000

Additional Material Required but not Supplied

1. Optical-grade qPCR tubes, plates, sealing films, and aerosol-resistant pipette tips.
2. qPCR primers and probes.
3. DNA or cDNA templates.

Precautions

1. Fully thaw qPCR Probe Set V2 before use.
2. This product contains polymerase. Please place it on ice when using it. It can be temporarily stored at 4 ℃ if used multiple times in a short period of time. Repeated freeze-thaw should be avoided .
3. Use the ROX reference dye according to the requirement of qPCR instrument to be used.
4. If applicable, use aerosol-resistant pipette tips to minimize contamination.
5. High quality DNA templates are recommended for optimal results.

Protocol

Important points before reaction setup:

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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. The volume of template should not exceed 10% of the final PCR reaction volume.
5. Always include a no template control (NTC) reaction.
6. Triplicates are recommended as technical replicates in real-time PCR reactions.

Recommended Reaction

Components	20 μL
4X qPCR Probe Buffer II(No dNTPs and Mg2+)	5 μL
Entrans *Taq* II DNA Polymerase	0.1-0.2 μL
Forward Primer (10 μM)	0.4 μL
Reverse Primer (10 μM)	0.4 μL
dNTPs (10 mM each)	0.25 μL
MgCl2 (50 mM)	1.2 μL
Probe (10 μM)	0.4 μL
50X ROX Dye (as required by instrument guidelines)	0.4 μL
DNA Template	2 μL (<50 ng)
Nuclease-free Water	to 20 μL

Note:

1. If the reaction system needs to be changed to 25 μL, the corresponding volume of reaction solutions in the above list can be increased by 1.25 times.

2. Fully thaw 4X qPCR Probe Buffer II at room temperature, and gently mix well without creating bubbles. Spin down briefly in a microcentrifuge to collect all contents at the bottom.

3. Calculate the required volume of each components based on the number of reactions to be set up and add extra 10% volume of each component to compensate pipette errors.

4. Add all the common reaction components (primers and probes) in a master mix and mix thoroughly.

5. Dispense appropriate volumes of reaction mix into qPCR plates, and carefully seal it with an optical sealing film.

6. Add templates or NTC into wells containing the qPCR reaction mix.

7. Centrifuge the qPCR plates (tubes) at 2500 rpm to collect all the contents at the bottom of wells. The samples are ready for thermocycling.

Recommended PCR Program

Step	Temp	Time	Cycles
Predenaturation	95℃	10 min	1
Denaturation	95℃	15 s	
Annealing and extension	60℃	30 s	40

Note ： 1. To ensure signal acquisition after extension, the extension temperature should be based on the Tm value of the primer probe.Line adjustment.

2. It is recommended that the shortest predenaturation time should not be shorter than 3 min, and the longest should not exceed 10 min; the shortest denaturation time during the cyclic reaction is not less than 5s, and the longest is not more than 15s; the cyclic reaction; the shortest

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extension time in the application is not less than 10s, and the longest can be based on the primer probes and signals used by yourself.The set needs to be adjusted by itself.

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 the Ct values between replicates should be <0.2 cycle. For most cases, the STDs of Ct values <0.5 cycle are acceptable.
3. The Ct value of a valid amplification should be less than the value of the NTC curve.

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