Entrans qPCR Probe Set

Catalog: RK21210

Size: 100 RXN / 500 RXN (20 μL/RXN)

Components:

Entrans *Taq* DNA Polymerase (5,000 U/ml)	RM21209
4X qPCR Probe Buffer II (No dNTPs and Mg^{2+})	RM21211
50X ROX Dye I	RM21465
50X ROX Dye II	RM21466
dNTPs (10 mM each)	RM20120
$MgCl_2$ (50 mM)	RM20144

Product Description

Entrans qPCR Probe Set is designed for probe-based qPCR reactions. It contains all components required for a rapid and reliable qPCR reaction except primers, probes and templates. When an intact probe binds to the complementary template DNA strand, the reporter dye and quencher are in proximity and the quencher absorbs the fluorescence emitted by the reporter dye. In PCR extension, the 5 '-3 'exonuclease activity of Taq polymerase cleaves the hybridized probe, separating the reporter from the quencher and releasing fluorescent signal. This kit includes an engineered version of Taq polymerase with antibody-mediated Hot-start capability to inhibit non-specific amplification at room temperature, while allowing high efficiency, sensitivity and reproducibility in qPCR amplification. Separated tubes of dNTPs and $MgCl_2$ are included in the kit, allowing customized qPCR reactions. The Entrans qPCR Probe Set is an ideal product for high-speed multiplex qPCR analysis.

Compatible Instruments

Reference Dye	Instruments
No ROX	Bio-Rad iCyclers/ CFX96/ CFX 384, Roche Light Cyclers® QIAGEN/Corbett Systems Rotor-Gene™ Systems Eppendor Mastercyclers®
ROX Reference Dye I	Applied Biosystems 7000/7300/7700/7900, Applied Biosystems StepOne™/StepOnePlus™, ,
ROX Reference Dye II	QuantStudio™, Applied Biosystems 7500/ ViiA7™ Stratagene Real-time Systems,

Product Components

Components	100 RXN	500 RXN
Entrans *Taq* DNA Polymerase (5,000 U/ml)	10 μL	50 μL
4X qPCR Probe Buffer II (No dNTPs and $MgCl_2$)	500 μL	1.25 mL x 2
50X Reference Dye I	44 μL	220 μL
50X Reference Dye II	44 μL	220 μL
dNTPs (10 mM each)	25 μL	75 μL
$MgCl_2$ (50 mM)	1.25 ml	1.25 ml

Storage

Upon receipt, store all components at -20℃.

Additional Materials Required but not Supplied

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

Precautions

1. Fully thaw 4X qPCR Probe Buffer II before use.
2. The 4X qPCR Probe Buffer II contains glycerin. Gently mix well before use without generating bubbles. Spin briefly to collect all the contents at the bottom. After use, return it to -20 °C immediately.
3. A Hot-start version of Taq polymerase is included in the set, allowing reaction setup at room temperature. After the initial thaw, it is stable at 4 °C for 1 week. Avoid multiple freeze-thaw cycles.
4. This product includes with a specially designed ROX reference dye for the fluorescent signal normalization. Use the ROX reference dye according to the requirement of qPCR instrument to be used.
5. If applicable, use aerosol-resistant pipette tips to minimize contamination.
6. High quality DNA templates are recommended for optimal results.

Protocol

Important points before reaction setup:

(1) A final primer concentration of 0.2 μM is recommended for most reactions. However, to optimize individual reactions, a primer concentration 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 templates to access standard curves 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.

Set up qPCR experiment:

1. Prepare the reaction mix:

(1) Fully thaw the 4X qPCR Probe Buffer II (No dNTPs and Mg^{2+}) at room temperature, and gently mix well without creating bubbles. Spin down briefly in a microcentrifuge to collect all contents at the bottom.

(2) 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.

(3) Add all the common reaction components in a reaction master mix and mix thoroughly.

(4) Dispense appropriate volumes of reaction mix into qPCR plates.

(5) Add templates or NTC into wells containing the qPCR reaction mix, and carefully seal it with an optical sealing film.

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

Component	Volume
4X qPCR Probe Buffer II (No dNTPs and $MgCl^2$)	5 μL
Entrans *Taq* DNA Polymerase (5,000 U/ml)	0.1 μL
Forward Primer (10 μM)	0.4 μL
Reverse Primer (10 μM)	0.4 μL
dNTPs (10 mM each)	0.25 μL
$MgCl_2$ (50 mM)	1.2 μL
Probe (10 μM)	0.4 μL
50X ROX (as required by instruments guideline)	0.4 μL or 0 uL
DNA Template	Up to 2 μL (<50 ng)
Nuclease-free Water	Up to 20 μL

2. Set up the qPCR cycling conditions

Step	Temperature	Time	Cycles
Polymerase activation	95℃	3 min	1
Denaturation	95℃	15 sec	40
Annealing and extension	60℃	30~34 sec*	

** The extension time should be adjusted to the minimum time required for data acquisition according to qPCR instrument guidelines used. (30 s for Applied Biosystems StepOnePlus™, 31 s for Applied Biosystems 7300, and 34 s for Applied Biosystems 7500)*

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 (R^2) 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 curves.

Related Products

Product	Catalog No.
ABScrpipt II RT Master Mix for qPCR	RK20402
ABScrpipt II RT Mix for qPCR with gDNA Remover	RK20403
ABScript II cDNA First-Strand Synthesis Kit	RK20400
2X Universal SYBR Green Fast qPCR Mix	RK21203
ABScript II One Step SYBR Green RT-qPCR Kit	RK20404
Entrans 2X qPCR Probe Master Mix	RK21208
Entrans qPCR Probe Kit	RK21209
ABScript II One Step RT-qPCR Probe Kit	RK20407