Entrans qPCR Probe Kit

Cat. No. : RK21209

Product components

Components	Component number	Size-1 100 RXN (20 μL/RXN)	Size-2 500 RXN (20 μL/RXN)
Entrans Taq DNA Polymerase (5,000 U/mL)	RM21209	10 μL	50 μL
4X qPCR Probe Buffer*	RM21210	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

Product Description

The Entrans qPCR Probe Kit is a specialized reagent kit for qPCR reactions based on the probe method. This product utilizes antibody-modified heat-activated Taq DNA polymerase for amplification, ensuring high amplification efficiency while significantly enhancing product specificity. It also features precise quantification, good repeatability, and a wide dynamic range. Through optimization of the buffer system, the product enables multiplex fluorescent quantification experiments and is applicable to multiple species, providing a powerful tool for interdisciplinary experimental needs.

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 RequiredbutnotSupplied

1. Optical-grade qPCR tubes, plates, sealingfilms, and aerosol-resistant pipettetips
2. qPCR primers and probes
3. DNA or cDNA templates

Precautions

1. Before use, fully dissolve the 4X qPCR Probe Buffer.
2. The 4X qPCR Probe Buffer contains glycerol. Before use, gently mix to avoid bubble formation; mix thoroughly and centrifuge before aliquoting. Store immediately at -20℃ after use.
3. This product contains polymerase. Please keep it on ice during use. It can be stored temporarily at 4℃ for short periods, but repeated freeze-thaw cycles should be avoided as much as possible.
4. Choose appropriate reference dyes according to the qPCR machine model when using.
5. For reaction solution preparation and aliquoting, sterile pipette tips should be used, and it is recommended to use tips with filters.
6. To increase the success rate of the reaction, it is recommended to use high-quality DNA templates.

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 PCR Reaction

1. Prepare the following reaction solution on ice (using a 20 μL reaction system as an example)

Components	Volume
4X qPCR Probe Buffer	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
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 25μL, simply increase the volume of all reaction solutions listed above by 1.25 times.

2. Thaw 4X qPCR Probe Buffer at room temperature, then place it on ice for later use. After complete thawing, gently vortex to mix thoroughly, avoiding bubble formation, and finally centrifuge briefly.

3. Calculate the required volume of the experiment mix and leave sufficient margin (generally more than 10%).

4. Precisely pipette liquids into clean PCR tubes or EP tubes, taking care to prevent liquid contamination and errors caused by manipulation.

5. Add the corresponding primers, probes, and template separately. After adding all components (excluding enzyme-free water, Mix, and ROX, etc.), gently mix by vortexing and finally centrifuge briefly.

6. Transfer the prepared reaction solution to a dedicated qPCR plate (tube) and carefully seal it with matching sealing consumables (be careful not to cause bubbles during transfer and try to avoid contact with the film).

7. Centrifuge the qPCR plate (tube) at 2500 rpm for 2-3 minutes to prepare for machine operation.

2.Set up the qPCR conditions

Step	Temp	Time	Cycles
Predenaturation	95℃	3 min	1
Denaturation	95℃	15 s	
Annealing and extension	60℃	30~34 sec*	40

*** 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 (R2) is >0.98, the template concentration data points are within the

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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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