Entrans 2X qPCR Probe Master Mix

Cat. No. : RK21208

Product components

Components	Component number	Size-1 100 RXN (20 μL/RXN)	Size-2 500 RXN (20 μL/RXN)
Entrans 2X qPCR Probe Master Mix*	RM21207	1 mL	1 mL X 5
50X ROX Dye I	RM21465	40 μL	200 μL
50X ROX Dye II	RM21466	40 μL	200 μL

*Including Hot Start Taq DNA polymerase, Mg^{2+}, dNTPs, etc.

Product Description

Entrans 2X qPCR Probe Master Mix is a specialized reagent for qPCR reactions based on the probe method. This product utilizes a heat-activated Taq DNA polymerase, which is chemically and antibody-modified, for amplification. It ensures high amplification efficiency while greatly enhancing specificity. Additionally, it 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.

This product is a 2X concentration premixed solution containing all components required for qPCR reactions, except primers, probes, and templates.

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
50X ROX Dye I	Applied Biosystems 7000/7300/7700/7900, Applied Biosystems Step One Plus
50X ROX 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. Fully thaw Entrans 2X qPCR Probe Master Mix before use.
2. The Entrans 2X qPCR Probe Master Mix contains glycerin. Mix gently before use without generating air bubbles. Spin briefly to collect all the contents at the bottom. After use, return it to -20℃ immediately.
3. A Hot-start version of Taq polymerase is included in the master mix, allowing reaction setup at room temperature. After first thaw, the master mix is stable at 4℃ for 1 week.
4. This product is equipped 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.

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Protocol

Important points before reaction setup:

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
Entrans 2X qPCR Probe Mater Mix	10 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 Entrans 2X qPCR Probe Master Mix 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℃	10 min**	1
Denaturation	95℃	15 s***	40
Annealing and extension	60℃*	30 s****	

*** To ensure signal acquisition after extension, the extension temperature should be adjusted according to the Tm value of the primers/probes.**

**** In general, the predenaturation time is recommended to be no less than 3 min and no more than 10 min.**

***** The denaturation time should be no less than 5 s and no more than 15 s.**

****** The extension time should be no less than 10 s, and the maximum duration can be adjusted according to the primers/probes used and the signal acquisition requirements.**

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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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