Entrans 2X qPCR Probe Master Mix with UDG V5

Cat. No. : RK21225

Product components

Components	Component number	Size-1 100 RXN (25 μL/RXN)	Size-2 500 RXN (25 μL/RXN)
Entrans 2X qPCR Probe Master Mix with UDG V5	RM21224	1.25 mL	1.25 mL*5
10X qPCR Enhancer	RM21222	250 μL	1.25 mL

Product Description

The Entrans 2X qPCR Probe Master Mix with UDG V5 is a specialized reagent tailored for probe-based qPCR reactions. This product utilizes a chemically and antibody-modified hot-start Taq DNA polymerase to ensure amplification while significantly improving specificity. This mix provides precise quantification, high amplification efficiency, excellent repeatability, and a broad reliable range. Its optimized buffer system facilitates the pre-mixing of primers, probes, and water, while the integrated UDG contamination prevention system effectively reduces the risk of false positives due to PCR product contamination.

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 ABI StepOne/StepOnePlus
ROX Reference Dye II	Applied Biosystems 7500/ViiA7™, QuantStudio™ Stratagene Real-time PCR Systems, Rotor-gene™ 3000，Strata gene

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. Ensure that the reagent is fully thawed before use. Gently mix before use to avoid creating bubbles, then thoroughly and centrifuge before use. After use, immediately return it to -20℃ storage.
2. This product contains polymerase. During use, keep it on ice. If multiple uses are required within a short period, it can be temporarily stored at 4℃. Repeated freeze-thaw cycles should be avoided as much as possible.
3. Choose an appropriate reference dye based on the qPCR machine model you are using.
4. When preparing and dispensing the reaction mixture, use sterile pipette tips, preferably those with filters.
5. To increase the success rate of the reaction, it is recommended to use high-quality DNA templates. If you need to pre-mix primers and probes for stability testing at different environmental temperatures, the final primer concentration can be adjusted between 0.4-1 μM.

Protocol

Important points before reaction setup:

(1) Ensure the correctness and specificity of primer design. Generally, a final primer concentration of 0.2 μM yields good results. If amplification efficiency is suboptimal, the primer concentration can be adjusted within a range of 0.1-1.0 μM.

(2) It is recommended that the length of the amplification product be in the range of 70-200 bp.

(3) Perform gradient dilution of the template and successively establish a standard curve.

(4) In a 25 μL reaction system, it is recommended to add 1 pg-50 ng of DNA as a template and design a NTC.

(5) To ensure the accuracy of the experimental results, it is recommended to perform each sample and control group in triplicate.

(6) For viral detection and more complex templates, you can add 250 μL of 10X qPCR Enhancer to 1.25 mL of Entrans 2X qPCR Probe Master Mix with UDG V5, mix thoroughly by shaking, or calculate and add according to the actual usage amount.

Recommended Reaction

Components	25 μL
Entrans 2X qPCR Probe Master Mix with UDG V5	12.5 μL
Forward Primer (10 μM)	0.5 μL
Reverse Primer (10 μM)	0.5 μL
Probe (10 μM)	0.5 μL
50X ROX Dye (as required by instrument guidelines)	0.5 μL
DNA Template	5 μL (<50 ng)
Nuclease-free Water	to 25 μL

Recommended Reaction（add enhancer）

Components	25 μL
Entrans 2X qPCR Probe Master Mix with UDG V5	12.5 μL
10X qPCR Enhancer	2.5 μL
Forward Primer (10 μM)	0.5 μL
Reverse Primer (10 μM)	0.5 μL
Probe (10 μM)	0.5 μL
50X ROX Dye (as required by instrument guidelines)	0.5 μL
DNA Template	5 μL (<50 ng)
Nuclease-free Water	to 25 μL

Note:

(1) Calculate the required volume of the reaction mixture and prepare an adequate surplus (generally, the surplus should be more than 10%).

(2) Accurately pipette the liquid into a clean PCR tube or EP tube, taking care to prevent contamination and minimize errors caused by handling.

(3) Add the appropriate primers, probes, and template. After adding all components (Nuclease-free water, MIX, and ROX), gently mix by shaking and then briefly centrifuge.

(4) Transfer the prepared reaction mixture into a qPCR plate (or tubes), and seal it. Ensure no bubbles are introduced during transfer, and avoid contact between the liquid and the sealing materials.

(5) Centrifuge the qPCR plate (or tubes) at 2500 rpm for 2-3 minutes, then send it (them) to the machine.

Recommended PCR Program

Step	Temp	Time	Cycles
UDG digestion	37℃	2 min	1
Predenaturation	95℃	3 min	1
Denaturation	95℃	10s	
Annealing and extension	60℃	30 s	40

Note ： (1) To ensure signal collection after extension, the extension temperature should be based on the Tm value of the primer probe.

(2) The pre-denaturation time can be set between 3 to 10 minutes. During the cyclic reaction, the denaturation time should be no less than 5 seconds and no more than 15 seconds. The extension time should be at least 10 seconds, with the maximum extension time adjustable based on the specific primers, probes, and signals used in your application. Adjust the settings as needed.

Data Analysis

1. A standard curve involves a linear regression analysis of the data, plotting Ct values versus against the the logarithm of the sample input concentration. If If the correlation coefficient (R2) is >0.98, the template concentration data points fall 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 less than 0.2 cycle. In most cases, the STDs of Ct values under 0.5 cycle are acceptable.
3. The Ct value of a valid amplification should be lower than that of the no-template control (NTC) .