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TAQMAN PROBE 2X qPCR SUPER MIX UDG V5 FS-T-72225

Description

TaqMan Probe 2X qPCR Super Mix UDG V5 ready-to-use reagent ideal for most quantitative Real-time PCR applications. containing all components except primers, probes and templates. This Super Mix includes Hot start Taq DNA polymerase modified both chemically and by antibody to inhibit non- specific amplification, which can guarantee high efficiency, high sensitivity and also high reproducibility in qPCR amplification. Is is optimized by UDG anti- pollution system.
It is bacterial DNA free for human and veterinary samples.
This Super Mix has additional capabilities for your gene expression analysis

For viral detection and more complex templates use 10xqPCR Enhancer

Kit Contents

Contents	CAT. N°	Size
TaqMan Probe 2X qPCR Super Mix UDG V5	FS-T-72225	*500 RX 5x 1,25ml
10 X qPCR Enhancer		1 vial
50x Rox Dye I (High Rox)		1 vial
50x Rox Dye II (Low Rox)		1 vail

PCR Machines requiring ROX dye

- **High Rox Dye:**
  ABI 7000, 7300, 7700, 7900HT and 7900HT Fast, StepOne, StepOne Plus:
- **Low ROX Dye:**
  ABI 7500, 7500 Fast, Viia 7, QuantStudio; Roche LightCycler; Stratagene Mx3000, Mx3005P and Mx4000 :

PCR Machines requiring no ROX Dye

BioRad: iCycler, MyiQ, MiQ 2, iQ5, CFX-96, CFX-384, MJ Opticon, Option2, Chromo4, MiniOpticon

Qiagen: Roto-Gene Q, Roto-Gene3000, Roto-Gene 6000 -
Roche:LightCycler 480,LightCycler 2.0

Additional Material Required but not Supplied

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

Applications

- Real-time PCR
- Gene expression profiling
- Gene knockdown verification
- Array validation

Note

Do not contaminate the TaqMan Probe 2x qPCR Super Mix with primers, probe and template DNA used in individual reactions. Thaw and mix all components thoroughly, spin down shortly and chill on ice.

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°C 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°C. 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 qPCR reaction,it is recommended to use high-quality DNA template the. If you need to premix primer and probes, the primer conc.should be 0,4-1µM.

Important Steps before reaction

(1) Ensure the correctness and specificity of primer design. Generally, 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 2X qPCR Taqman Probe Super Mix UDG V5, mix thoroughly by shaking, or calculate and add according to the actual usage amount.

Protocol

1- The following table shows recommended component volumes:

Reaction Conditions

	25 µl
TaqMan Probe 2X qPCR Super Mix UDG V5	12,5 µl
ROX Dye (50X) *(optional)	0.5-0.6 µl
10um Forward Primer	0.5~0.6 µl
10 um Reverse Primer	0.5~0.6 µl
Probe (10µm)	0.5~0.6 µl
Template	5 µl <50ng
Water RNase Free	up to 25 ul

***Please note "Use of the ROX Reference Dye"**

2.Ensure reactions are mixed thoroughly by pipetting or gentle vortexing followed by a brief spin in a microcentrifuge.
3.Transfer tubes on ice into a thermal cycler pre-warmed. The following table shows recommended cycling conditions
4. 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.

Reaction Conditions

	25 µl
TaqMan Probe 2X qPCR Super Mix UDG V5	12,5 µl
10X qPCR Enhancer**	2,5 µl
ROX Dye (50X) *(optional)	0.5-0.6 µl
10um Forward Primer	0.5~0.6 µl
10 um Reverse Primer	0.5~0.6 µl
Probe (10µm)	0.5~0.6 µl
Template	5 µl <50ng
Water RNase Free	up to 25 ul

****use the 10X qPCR Enhancer For viral detection and more complex templates**

Store: at-20°C , avoid freezing and thawing cycles.

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Program your instrument as instructions below

Standard Cycling PCR Conditions

Step	Temp	Time	Cycles
UDG Digestion	37℃	2 min	1
Predenaturation**	95℃	3 min	1
Denaturation	95°C	10 sec	40
Annealing and **extension** *	60℃	30 sec	40

Fast Cycling Program PCR Conditions

Step	Temp	Time	Cycles
UDG Digestion	37℃	2 min	1
Predenaturation**	95℃	3 min	1
Denaturation	95°C	5-9 sec	40
Annealing and **extensio** *	60℃	30 sec	40

** It is recommended that the shortest **predenaturation** time should not be shorter than 30s, 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 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.

* To ensure signal acquisition **after extension**, the extension temperature should be based on the Tm value of the primer probe.

The setting needs to be adjusted by the researcher

Data Analysis

1.*A standard curve envolves a linear regression analysis of the data, plotting as the Ct values versus against the logarithm of the sample input concentration.*
If the standard curve 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 <0.2 cycle.*
In most cases, the STDs of Ct values less than 0.5 cycle are acceptable.
3.*The Ct value of a valid amplification should be lower than that of the No-Template Control (NTC)*

Quality Control Analysis
Sensitivity and reproducibility in real-time PCR are tested in parallel reactions containing 10-fold dilutions of nucleic acid template

For Research Use Only

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