FISHER MOLECULAR BIOLOGY

TAQMAN PROBE qPCR 2X MASTER MIX (UDG) FS-T-50217

Description

TaqMan Probe qPCR 2X Master Mix with UDG is a ready-to-use reagent for probe-based qPCR reactions, containing all components except primers, probes and templates. This master 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.
At the same time, and has joined the UDG anti- pollution system. The optimization of the Buffer system allows the product to perform multiple fluorescence quantitative experiments, and it is suitable for multiple species and provides a powerful tool for multi-disciplinary experimental need

Kit Contents

Contents	CAT. N°	Size
TaqMan Probe qPCR 2X Master Mix with UDG	FS-T-50217	500 RX
Rox Dye I (high Rox) 50X		1 Vial
Rox Dye II (low Rox) 50X		1 Vial

1ml = 100 Reactions

ROX dye - Real Time Machines:

High Rox Dye: ABI 7000, 7300, 7700, 7900HT and 7900HT Fast, StepOne,stepOne Plus:
Low ROX Dye: ABI 7500, 7500 Fast, Viia 7, QuantStudio; **Qiagen**: Roto-Gene Q, Roto-Gene3000, Roto-Gene 6000

No ROX Dye – Real Time Machines

BioRad: iCycler, MyiQ, MiQ 2, iQ5, CFX-96, CFX-384, MJ Opticon, option2, Chromo4, MiniOpticon **Roche**:LightCycler 480, LightCycler 2.0 **Eppendorf**: Mastercycle realplex - **Illumina**: Eco RealTime PCR System Cepheid: SmartCyler

Applications

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

Component	50 rxn	500 rxn
Taqman Probe 2X qPCR Probe Master Mix with UDG*	500 µl	4 x1,25 mL
Rox Dye I (High Rox) conc.50x	22 µL	2x100 µL
Rox Dye II (Low Rox) conc.50x	22 µL	2x100 µL

**Contain hot-start Taq DNA Polymerase,UDG, Mg2+, dNTPs et. al.*

Note

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

Additional Material Required but not Supplied

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

Storage: Upon receipt, store all components at –20°C.

Precautions

1.Fully thaw TaqMan 2X qPCR Probe Master Mix with UDG before use.
2.The TaqMan 2X qPCR Probe Master Mix with UDG 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°C immediately.
3.A Hot-start version of Taq polymerase is included in the master mix, allowing reaction. After first thaw, the master mix is stable at 4 °C for 1 week
4.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.

Important points before set up:

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.

Set up: Prepare the reaction mix.

1.Fully thaw the TaqMan Probe 2X qPCR Probe Master Mix with UDG at room temperature, and gently mix well without creating bubbles. Spin down briefly in a microcentrifuge to collect all contents at the bottom.

Reaction Conditions

Reagents	20 µl reaction
TaqMan Probe qPCR 2X Master Mix with UDG	10.0µl
(10 µm) Forward Primer	0.4µl
(10 µm) Reverse Primer	0.4µl
Fluorescence Probe(10 µm)	0.4µl
Rox Dye (50X) optional*	0.4µl
DNA Template**	0.4µl(<50ng)
Water RNase Free	Up to 20µl

***Please note "Use of the ROX Dye on Real Time Machines"**

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 (primers and probes) in a master mix and mix thoroughly.
4.Dispense appropriate volumes of reaction mix into qPCR plates, and carefully seal it with an optical sealing film.
5.Add templates or NTC into wells containing the qPCR reaction mix.
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.

PCR Conditions

Step	Tem p (°C)	Time	Cycle
UDG	37°	2 min	1
Predenaturation	95°	*20 sec.~5min.	1
Denaturation	95°	5 sec.	40
Annealing and Extention	55°	10 sec.	

**To ensure signal acquisition after extension, the extension temperature should be based on the Tm value of the primer probe.Line adjustment.*
*** It is recommended that the shortest pre-denaturation 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. The set needs to be adjusted by itself.*

For Research Use Only

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