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TAQMAN PROBE qPCR 2X GENOTYPING MASTER MIX (UDG) FS-T-72212

Description

TaqMan Probe qPCR 2X Genotyping Master Mix with UDG is a ready-to-use reagent for SNP genotyping probe-based for multiplexing 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.

It contains dUTP/UDG anti-contamination system, where thermolabile UDG rapidly degrades uracil-containing DNA at room temperature. During the 95°C pre-denaturation step, the thermolabile UDG is quickly inactivated, preventing carryover contamination and ensuring the accuracy of genotyping. This product offers advantages such as accurate genotyping of low-concentration templates and excellent reproducibility.

Kit Contents

Contents	CAT. N°	Size
TaqMan Probe qPCR 2X Genotyping Master Mix with UDG*	**FS-T-72212**	**5ml/500 RX**
Rox Dye I (high Rox) 50X		**1 Vial**
Rox Dye II (low Rox) 50X		**1 Vial**

1ml = 100 Reactions

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

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- Stratagene Real Time PCR System

No ROX Dye – Real Time Machines

BioRad: iCycler, MyiQ, MiQ 2, iQ5, CFX-96, CFX-384, MJ Opticon, 4, MiniOpticon Roche:LightCycler 480, LightCycler 2.0, Quiagen/Corbett

Applications

- Real-time PCR/Genotyping / PCR samples from human or animal sources

Note

Do not contaminate the TaqMan Probe 2X qPCR Genotyping 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 Probe 2X qPCR Genotyping Master Mix with UDG before use.

2.The TaqMan Probe 2X qPCR Genotyping Master Mix with UDG should be gently mixed before use to avoid generating bubbles. Mix and briefly centrifuge prior to reaction setup. After use, return immediately to the -20°C storage.

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.

Experimental Preparation:

1.EP tubes, PCR tubes, pipettes, pipette tips, and ice boxes.

2.PCR probes, primers, and templates.

3.Tubes or plates specifically for quantitative PCR, along with sealing consumables

4.Reagents to be prepared by the user: DNA templates, primers, probes, and nuclease-free H_2O.

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 Genotyping Master Mix with UDG	10 µl
(10 µm) Forward Primer	0.25-0.5µl
(10 µm) Reverse Primer	0.25-0.5µl
Fluorescence Probe(10 µm)	0.25-0.5µl
Rox Dye (50X) optional*	0.2 µl
DNA Template**	100pg/100 ng
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.Transfer the appropriate volumes of reaction mix into qPCR plates, and carefully seal it with an optical sealing film, avoiding bubbles formation and preventing the liquid from contacting the sealing surface

5.Add templates or NTC into wells containing the qPCR reaction mix.

6.Centrifuge the qPCR plates (tubes) at 500 rpm before loading it into the instrument.

PCR Conditions

Step	Tem p (°C)	Time	Cycle
Predenaturation	95°	5min.	1
Cycling	95° 60°	5 -15 s 15-30 s	40-45
Final signal acquisition	60°	1 min.	

Notes:

* UDG enzyme is heat-sensitive and active at room temperature. It begins acting before the PCR program starts and is irreversibly inactivated during the pre-denaturation step.

* Generally, the pre-denaturation time is recommended to be no shorter than 3 minutes and no longer than 10 minutes. During cycling, the denaturation time should be no shorter than 5 seconds and no longer than 15 seconds, while the extension time should be no shorter than 10 seconds and can be adjusted according to the requirements of the primers, probes, and signal collection.

* If abnormal genotyping results occur, fluorescence collection can be collected during the annealing and extension steps to monitor signals changes throughout amplification.

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TROUBLESHOOTING

Unusual Amplification Curves

a.Unsmooth amplification curve: The signal is too weak. Increase the template input amount and repeat the experiment.

b.Sudden drop in individual amplification curves: This may be due to bubbles in the reaction tube. As the temperature rises, the bubbles burst, causing a sudden decrease in the fluorescence signal detected by the instrument. Ensure thorough centrifugation of the reaction mix and avoid introducing bubbles during handling and pipetting.

c.Upward drift in amplification curves: The instrument typically sets the baseline from cycles 3-15, which may not fit actual conditions. Other possible causes include degraded template/primers or inefficient primer amplification. Adjust the baseline according to the actual amplification conditions. Before running qPCR, confirm template concentration and primer quality.

No Amplification Curves after Reaction

a.Signal collection not set: Ensure that fluorescence collection is enabled , typically during the annealing/extension step.

b.Insufficient number of reaction cycles: The cycle number is generally set to 40 – 50. However, it should be noted that excessive cycles may increase background signals and reduce data reliability.

c.Template or primer degradation: For templates/primers stored long-term, verify concentration and integrity to rule out degradation. .

d.Too low template input: Increase the amount of template input.

Ct Value Too Late

a.Low amplification efficiency: Optimize the reaction program and system; redesign primers if necessary.
b.Excessive amplicon length: Target length should generally be 70–200 bp.
c.PCR inhibitors in template: Dilute template further or re-extract to remove inhibitors.
d.Too Low Template Input: Increase the amount of template input.

Quality Control Analysis

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

Store at: -20° upon receipt -. Avoid freeze and thaw cycles

NTC Shows Amplification

a.Contamination: Replace Mix and water, then repeat the experiment. Prepare reactions in a laminar flow cabinet to minimize aerosol contamination
b**.Non-Specific PCR Products**: If contamination is excluded, redesign primers to improve specificity.

Inconsistent Results

a.Pipetting inaccuracy: Use calibrated pipettes and increase microreagents volume where feasible.

b.**Temperature variation across wells**: Regularly calibrate the qPCR instrument.
c.Inconsistent threshold setting: Apply the same threshold across different runs and plates.
d.Too low template input: Increase the amount of template input.

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