NucleoGene
qPCR Probe Master Mix (2X)
Instructions for Use

Release Date— 01.08.2022

Store at -20°C

REF

For research use only.
Not suitable for diagnostic use.
For professional use only.

NGMM004 20- µ L x 200 rxn
20- µ L x 500 rxn
20- µ L x 1000 rxn

Description

NucleoGene qPCR Probe Master Mix (2X) allows you to make the best hotstart PCR applications with Taq polymerase combined with an chemical modification. The special additives in its content help to eliminate foaming ofthe mixture resulting from vortexing, so that valuable samples are retained with fewer errors. Specially developed buffer chemistry works in harmony with almost all of your reaction designs. NucleoGene qPCR Probe Master Mix (2X) reagents contain an inhibitor neutralizing additive that inhibits several types of samples like clinical, plant, or environmental samples or complex food matrices, providing reliable qPCR assay performance for environmental analysis, GMO (genetically modified organism) analysis and analysis for clinical samples (stool including). In addition, NucleoGene qPCR Probe Master Mix (2X) reagents are optimized for use in single-step RNA analyses and different RNA analyses.

Instrument Compatibility

Different real-time PCR systems employ different strategies for the normalization of fluorescent signals and correction of well-to-well optical variations. It is important to match the appropriate reference dye to each specific optical detection system. NucleoGene qPCR Probe Master Mix (2X) does not contain an internal reference dye. Please check if the product is suitable for your device.

Components

NucleoGene qPCR Probe Master Mix (2X):
2X reaction buffer containing optimized concentrations of MgCl2, dNTPs (dATP, dCTP, dGTP, dUTP), hot-start DNA polymerase, inhibitory inactivators and stabilizers.

Storage andStability

Store components in a constant temperature freezer at -25°C to -15°C protected from light up on receipt. Repeated freezing and thawing does not affect PCR performance. For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form.

GuidelinesforqPCR

The design of specific primers and probes is a critical parameter for successfull real-time PCR. Using computer aided primer design programs may make it possible for you toget the best results. For best results, the size of the amplicon should be limited to 65 - 200 bp. Optimal results are usually performed with a primary concentration of 100 to 900 nM. The final concentration of 300 to 400 nM primer and 100 to 250 nM probe is effective for most applications. Increasing the concentration of primer that initiates the synthesis of the target strip complementary to the probem a improve the fluorescence signal for some primary / probe systems. It is recommended to prepare a reaction cocktail to reduce pipetting errors and maximize test sensitivity. Combine the reaction cocktail with all the necessary components except the sample template (genomic DNA or cDNA) and distribute them in equal amounts to each reaction tube. Add the DNA template to each reaction as the last step. The addition of samples using 2 to 5 uL of volume will increase test sensitivity.

Suggested input quantities of template are: cDNA corresponding to 1 pg to 150 ng of total RNA; 10 pg to 500 ng genomic DNA. After sealing each reaction, vortex gently to mix contents. Centrifuge briefly to collect components at the bottom of the reaction tube.

Reaction Assembly

Component	Volume for 20 uL rxn.	Final Concentration
NucleoGene Probe Master Mix (2X)	10 uL	1X
Forward Primer	Variable	100-900 nM
Reverse Primer	Variable	100-900 nM
Probe	Variable	100-250 nM
Nuclease Free Water	Variable	
Template DNA or cDNA	2-5 uL	
Final Volume (uL)	20 uL	

Real Time PCR Protocol

Standard Cycling

Initialdenaturation	95ºC 2 min 30 sec
Denaturation	95ºC 15s
Annealing/ Extension	55-60ºC, 40s

***PCR cycling (30-45 cycles)**

The appropriate step for fluorescent data collection varies for different probe types. Data collection for the hydrolysis probe assays (TaqManprobe) should be performed at the end of the extension step. Use the annealing step for data collection in hybridization probe experiments (HybProbe® FRET hybridization probes, Molecular Beacons, Solaris® qPCR Assays, Scorpions® primers, etc.). Detection probe chemistry end point analysis should be performed at an appropriate temperature.

The optimal initial denaturation time of the DNA polymerase depends on the DNA template and affects the qPCR efficiency and sensitivity. Reproduction of genomic DNA or plasmid DNA targets may take 10 minutes at 95° C to completely denature and fragment the template. The single stranded DNA template, such as the short double stranded DNA template (PCR product) or cDNA, may require as little time as 1 to 30 seconds at 95 ° C.

The extension time depends on the amplicon length and the minimum data collection time requirement for your qPCR. As a general starting point, you can use 30 seconds at 60 ° C. Some test designs and or detection

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chemicals may require a 3-stage cycling protocol for optimum performance. For any primer set and Real Time PCR device, the optimum annealing temperature and duration may need to be specifically determined.

Quality Control

Kit components are free of contaminating DNase and RNase. NucleoGene qPCR Probe Master Mix (2X) is functionally tested in qPCR. Kinetic analysis must demonstrate linear resolution oversix orders of dynamic range (R2 > 0.990) with a 2-fold discrimination of starting template and a PCR efficiency>95%.

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