NucleoGene qPCR SYBR Green 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.

NGMM007 20- µ L x 200 rxn
20- µ L x 500 rxn
20- µ L x 1000 rxn

Description

Nucleogene qPCR Sybr Green Master Mix allows you to make the best hotstart PCR applications with Taq polymerase combined with an ultra-pure antibody. The special additives in its content help to eliminate foaming of the 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 Sybr Green Master Mixreagents 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 Sybr Green Master Mixreagents 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 Sybr Green Master Mix does not contain an internal referencedye. Please check if the product is suitable for your device.

Composition

Nucleogene qPCR Sybr Green Master Mix (2X):
2X reaction buffer containing optimized concentrations of MgCl2, dNTPs (dATP, dCTP, dGTP, dUTP), hot start DNA polymerase, SYBR Green Dye, inhibitory inactivators, and stabilizers.

Applications

- High throughput Real Time PCR.
- Routine Quantitative or Qualitative qPCR with highre producibility.
- Real Time RT-PCR.

Storage andStability

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

E-mail: info@nucleogene.com
Web: www.nucleogene.com

Guidelines for qPCR

The design of specific primers is a critical parameter for successful Real-Time PCR. Using computer-aided primer design programs may make it possible for you to get 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 primer concentration of 100 to 900 nM. The final concentration of 300 to 400 nM primer is effective for most applications. Increasing the concentration of primer that initiates the synthesis of the target strip complementary may improve the fluorescence signal for some primer 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 qPCR Sybr Green Master Mix (2X)	10 uL	1X
Forward Primer	Variable	100-900 nM
Reverse Primer	Variable	100-900 nM
Nuclease Free Water	Variable	
Template DNA or cDNA	2-5 uL	Variable
Final Volume (uL)	20 uL	

Real Time PCR Protocol

	Standard Cycling	3-Step Cycling
Initialdenaturation	95ºC 2 min 30 s	95ºC 2 min 30 s
Denaturation	95ºC, 15 s	95ºC, 15 s
Annealing	55 to 65ºC 40s	55 to 65ºC 40 s
Extension/elongation		68 to 72ºC, 10s

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

***Melt Curve (dissociation stage) Refer to instrument instructions (optional)**

Full activation of Hot StartTaq DNA polymerase occurs within 10 min at 95° C. Initial denaturation times greater than 10 minutes are usually not required

when amplifying cDNA template. However, amplification of genomic DNA or supercoiled plasmid DNA targets may benefit from aprolonged initial denaturation step (15 min) to fully denature and fragment the template. This minimizes the potential for renaturation of longfragments and/or repetitive sequence regions that can impair replication of the target sequence by the PCR process.Some primer sets may require a 3-step cycling protocol for optimal performance. Optimal annealing temperature andtime may need to beempirically determined for any given primer set. A 68 to 72° C extension step of 30 seconds is suitable for most applications. However, amplicons-greater than 200 bp may require longer extension times. The use of an elevated temperature (80° C) for data collection is not recommended.While this technique can be used to mask the detection of primer-dimer and/or other non-specific products, it does little to improve assayspecificity or sensitivity and is not a substitute for effective primer design.

Quality Control

Kit components are free of contaminating DNase and RNase. Nucleogene qPCR Sybr Green Master Mixis functionally tested in qPCR. Kinetic analysis must demonstrate linear resolution over six orders of dynamic range (R2 > 0.990) with a 2-fold discrimination of starting template and a PCR efficiency >95%.

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