Lolina A/S

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Product Information

Product name	Lolina® Advanced qPCR SYBR Master Mix
Cat.No.	NaM601001
Size	1 mL/5×1 mL/50×1 mL/100×1 mL
Storage and shipping	1. The product is shipped with ice pack. 2. The product can be stored at -15℃ ~ -25℃ for 18 months. 3. The product contains fluorescent dyes, so it is necessary to avoid strong light irradiation when storing or preparing the reaction system.
Application equipment	**ABI:** 7500, 7500 Fast, ViiA7, QuantStudio1, 3 and 5, QuantStudio 6,7, 12k Flex; **Stratagene:** MX3000P, MX3005P, MX4000P; **Bio-Rad:** CFX96, CFX384, iCycler iQ, iQ5, MyiQ, MiniOpticon, Opticon,Opticon 2, Chromo4; **Eppendorf:** Mastercycler ep realplex, realplex 2 s; **Qiagen:** Corbett Rotor-Gene Q, Rotor-Gene 3000, Rotor-Gene 6000; **Roche Applied Science:** LightCycler 480, LightCycler 2.0, Lightcycler 96; **Thermo Scientific:** PikoReal Cycler; **Cepheid:** SmartCycler; **Illumina:** Eco qPCR

Product description

Lolina® advanced qPCR SYBR Master Mix is a pre-solution for 2× real-time quantitative PCR amplification. It has the characteristics of high fluorescence intensity, high sensitivity and specificity, and high amplification yield. It is blue in color and has the function of sample addition tracer. The core component Lolina® Taq DNA polymerase uses an antibody method to hot-start, which can effectively suppress non-specific amplification caused by primer annealing during sample preparation. At the same time, the formula adds a factor to improve the PCR reaction amplification efficiency and a promotion factor to balance the amplification of genes with

different GC contents (30~70%), so that quantitative PCR can obtain a good linear relationship in a wide quantitative range.

Operate

qPCR reaction System

Components	Volume μL[c)]	Volume μL[c)]	Final Conc.
Lolina® Advanced qPCR SYBR Master Mix	10	25	1 ×
Forward Primer (10 μmol/L)[a)]	0.4	1	0.2 μmol/L
Reverse Primer (10 μmol/L)[b)]	0.4	1	0.2 μmol/L
DNA/cDNA template[b)]	-	-	-
ddH_20	Up to 20	Up to 50	-

[Note]:

a) Primer concentration: The final primer concentration is 0.2 μmol/L, and can also be adjusted between 0.1 and 1.0 μmol/L as appropriate.

b) If the template is undiluted cDNA stock solution, the volume used should not exceed 1/10 of the total volume of the qPCR reaction. The optimal amount of template added is to ensure that the Ct value obtained by amplification is within 20-30 cycles.

c) It is recommended to use 20 μL or 50 μL to ensure the effectiveness and reproducibility of target gene amplification; mix thoroughly before use on the machine to avoid excessive bubbles caused by vigorous shaking.

Reaction program

Cycle step	Temp.	Time	Cycles
Initial denaturation	95 °C	2 min	1
Denaturation	95 °C	10 sec	40
Annealing/Extension[a)]	60 °C	30 sec[b)]	
Melting curve stage[c)]	Instrument Defaults		1

[Note]:

a) Annealing temperature and time: Please adjust according to the length of primer and target gene.

b) Fluorescence signal acquisition: Please set the experimental procedure according to the requirements in the instructions for use of the instrument. The time setting of several common instruments is as follows:

20 sec：Applied Biosystems 7700, 7900HT, 7500 Fast

31 sec：Applied Biosystems 7300

32 sec：Applied Biosystems 7500

c) Melting curve: The instrument default program can be used normally.

Primer Design Guide

1. The recommended primer length is about 25 bp. The optimal length of the amplified product is 150bp, and can be selected from 100 bp to 300 bp.

2. The difference in Tm values of forward primer and reverse primer should not exceed 2 °C. The optimal primer Tm value is 60 ℃ - 65 ℃.

3. The primer bases should be distributed evenly to avoid 4 consecutive identical bases, and the GC content should be controlled at around 50%. The last base at the 3' end is preferably G or C.

4. It is best to avoid complementary sequences of more than 3 bases within the primer or between the forward and reverse primers.

5. Primer specificity needs to be checked using the NCBI BLAST program, to avoid non-specific complementarity of more than 2 bases at the 3' end of the primer.

6. The designed primers need to be tested for amplification efficiency. Only primers with the same amplification efficiency can be used for quantitative comparative analysis.

Notes

1. For your safety and health, please wear lab coats and disposable gloves for operation.

2. After thawing, the Master Mix may appear flocculent or white precipitate. Dissolve it slowly by hand and mix it gently by inverting it up and down until the solution is clear. It will not affect the performance of the reagent.

3. It is recommended to use our company's cDNA synthesis kit (Cat. No.: NaM502002) to effectively remove residual genome from RNA samples.

4. If electrophoresis is required, in order to obtain clear bands, it is recommended to dilute the qPCR product 20 - 30 times before electrophoresis.

5. This product is for research use **ONLY**!