SYBR Green qPCR Mix (Low Rox+)

NB-03-0185 (1ml)
NB-03-0186 (1mlx5)

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Components

Component	NB-03-0185	NB-03-0186
SYBR Green qPCR Mix (Low Rox+)	1ml	1mlx5
Nuclease-free Water	1ml	1mlx5

Storage

This reagent can be stored at 4°C for 2 months and protected from light. For longer storage, it should be kept at -20°C and protected from light.

Description

SYBR Green qPCR Mix (Low Rox+) is designed for high-performance, high-throughput, real-time PCR. The kit contains a Taq DNA polymerase engineered through a process of molecular evolution. The result is a unique enzyme, specifically designed for qPCR using DS Green I dye chemistry.

SYBR Green qPCR Mix (Low Rox+) is a convenient premix of the components (except primers, template, and water) necessary to perform real-time polymerase chain reaction (PCR) using DS Green I dye with enhanced sensitivity and specificity. The DS Green dye binds to double-stranded (ds) DNA, thus providing a fluorescent signal that reflects the amount of dsDNA product generated during PCR. This reagent is used in amplification and detection of DNA in qPCR on ABI real-time instruments that support normalization with Rox reference dye at a final concentration of 50 nM.

Applications

- Gene expression analysis
- Low copy gene detection
- Microarray validation
- Gene knockdown validation

Features

- This reagent can be used in ABI Real-time systems that require low concentration of Rox reference dye.
- Hot Start technology with anti-Taq DNA polymerase antibodies enables high specificity and reproducible amplification.

Composition of the 2xDS Green qPCR Mix

100 mM KCl, 5 mM MgCl2, 400 µM dNTPs, 0.1 U/µl Hot Start Taq DNA Polymerase, 1x DS Green, 0.2% Rox reference dye and other optimized buffer components.

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Instrument Guide Table

Instrument	Rox Reference Dye
ABI 5700, 7000, 7300, 7700, 7900HT Step One™, and Step One Plus™	High Rox$^+$(Cat# NB-03-0187, NB-03-0188)
ABI 7500 Rox Low Stratagene Mx3000P®, Mx3005P™, and Mx4000®	Low Rox$^+$ (Cat# NB-03-0185, NB-03-0186)
Rotor-Gene™; DNA Engine Opticon™, Opticon® 2, and Chromo 4™ Real-Time Detector; No Rox Mastercycler® ep realplex, Smart Cycler®, Roche LightCycler® 480, Bio-Rad CFX96	No Rox (Cat# NB-03-0117, NB-03-0118)

Protocol

This protocol is intended for use with the ABI 7500 Rox Low, Stratagene Mx3000P®, Mx3005PTM, and Mx4000®. This kit is compatible with instruments that needs low concentration of Rox Reference Dye (50 nM).

1. Preparation of reaction solution

Add all the solution in a thin walled PCR tube on ice.

For a total 20µl reaction volume

Component of sample	Volume	Final concentration
2X DS Green qPCR Mix (Low Rox$^+$)	10 µl	1X
Forward Primer (10 µM)	0.4 µl	0.2 µM
Reverse Primer (10 µM)	0.4 µl	0.2 µM
Template DNA	variable	(<1ng/µl)
Water, nuclease-free	to 20 µl	–

Note

The primer concentration can be further optimized, if needed. The optimal range for primers is 0.1~1µM. The Template DNA concentration can be further optimized by gradient dilution. The concentration is generally <1ng/µl

2. Setup the plate

- Transfer the appropriate volume of reaction mixture to each well of a PCR tube/plate. Reaction volumes may be scaled down from 20 µl to 10 µl if low volume tubes/plates are used.
- Cap or seal the reaction tube/plate and centrifuge briefly.

3. Preform qPCR using the following thermal cycling conditions.

Initial Denaturation	95℃	20 sec-3 min*	Hold
Denature	95℃	5 sec **	40 Cycles
Anneal/Extend***	60℃	>20 sec	
Melting curve analysis			

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*20 sec at 95ºC is sufficient time for enzyme activation, however optimal denaturation of complex targets may require up to 3 min denaturation.
**Select minimum time (not less than 20 sec) according to instrument user guide. ***For 3 step cycling protocols, anneal at optimal annealing temperature for 20 sec followed by 5 sec extension and data acquisition at 72ºC.

4. Analyze the results

Data analysis varies depending on the instrument used. Please refer to your instrument user guide for information.

Important Notes

Template

Genomic DNA, plasmid DNA, or cDNA can be used as template. For optimal quantitative results use up to 20ng of genomic DNA or plasmid DNA per 20 µl reaction (for smaller volumes, the amount of template should be decreased equivalently). Using greater amounts of template may reduce the maximum fluorescence signal and linearity of standard curves due to binding of the DS Green I dye to the template. For two-step RT-PCR, use either undiluted or diluted cDNA generated from up to 1µg of total RNA. The volume of the cDNA added (from the RT reaction) should not exceed 10% of the final PCR volume (e.g., for a 20µl qPCR reaction, use up to 2.0µl of undiluted cDNA).

Primers

Careful primer design and purification (HPLC-purified primers are recommended) is particularly important in order to minimize loss in sensitivity due to the production of nonspecific amplification products in DS Green I-based qPCR. This effect becomes more prominent at low target concentrations. To maximize the sensitivity of the assay, use the lowest concentration of primers that can be used without compromising the efficiency of the PCR reaction (50-400nM of each primer). For optimal results, design primers that amplify PCR products 60-400 bp in length. The primers should exhibit a melting temperature (Tm) of approximately 60ºC, to take advantage of two-step cycling. If performing real-time two-step RT-PCR, we recommend designing primers specifically for amplification of cDNA derived from mRNA. This prevents amplification of contaminating genomic DNA and inaccurate quantification of mRNA.

Melting Curve Analysis

Following real-time qPCR, melting curve analysis should always be performed to identify the presence of primer-dimers and analyze the specificity of the reaction. Program your thermocycler according to the instructions provided.

DS Green I

SYBR Green qPCR Mix (Low Rox+) contains an elevated, optimized concentration of the fluorescent dye, DS Green I. High signal intensities are achieved as a result of increased tolerance to high concentrations of DS Green I by the engineered, novel Dongsheng DS DNA Polymerase. DS Green I binds all double-stranded DNA molecules, emitting a fluorescent signal on binding. The excitation and emission maxima of DS Green I are at 494 nm and 521 nm, respectively, which are compatible with use on any real-time cycler.

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Magnesium chloride

The MgCl2 concentration in SYBR Green qPCR Mix (Low Rox+) is optimized for most primer combinations. You do not need to add additional MgCl2 to the mix to get efficient and specific PCR.

Guidelines for preventing contamination of qPCR reaction

During qPCR more than 10 million copies of template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and amplicons that may be present in the laboratory environment. General recommendations to lower the risk of contamination are as follows:

- Prepare your DNA sample, set up the qPCR mixture, perform thermal cycling and analyze PCR products in separate areas.
- Set up aPCR mixtures in a laminar flow cabinet equipped with an UV lamp.
- Wear fresh gloves for DNA purification and reaction set up.
- Use reagent containers dedicated for PCR. Use positive displacement pipettes, or use pipette tips with aerosol filters to prepare DNA samples and perform PCR set up.
- Always perform “no template control” (NTC) reactions to check for contamination

Quality Control

The absence of endodeoxyribonucleases, exodeoxyribonucl- eases and ribonucleases is confirmed by appropriate quality tests. Functionally tested in amplification of a single-copy gene from human genomic DNA.

Product Use Limitations

SYBR Green qPCR Mix (Low Rox+) is sold exclusively for research purposes and in vitro use. Neither the product, nor any individual components, was tested for use in diagnostic applications or for drug development, nor is it suitable for administration to humans or animals. Please refer to the MSDS, available upon request.

FOR RESEARCH USE ONLY

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