version: 1.0

Cat. No: P2091a, 400 rxn/20μl reaction
P2092a, 2000 rxn/20μl reaction

SYBR® Green qPCR Mix (Low ROX+)

For research use only

Components

Component	P2091a	P2092a
2X SYBR® Green qPCR Mix (Low ROX+)	1 ml	1 ml × 5
Nuclease-free Water	1 ml	1 ml × 5

Storage

This reagent can be stored for 2 months at 4°C 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 Taq DNA Polymerase engineered through a process of molecular evolution. The result is a unique polymerase that specifically designed for qPCR using SYBR® Green I chemistry dye.

2X SYBR® Green qPCR Mix (Low ROX+) is a convenient premix of the components (except primers and DNA template) that necessary to perform real-time polymerase chain reaction (PCR) using SYBR® Green I dye with enhanced sensitivity and specificity. The SYBR® Green I dye binds to double-stranded DNA (dsDNA), thus providing a fluorescent signal that reflects the amount of dsDNA products generated during PCR.

Applications

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

Features

• Compatible with many Real-time systems which requires low concentrtion of ROX reference dye to calibration
• Hot-start technology brings high specificity and reproducible amplification

Composition of the 2X SYBR Green qPCR Mix (Low ROX+)

100mM KCl , 5mM $MgCl_2$, 400μM dNTPs, 0.1U/μl Hotstart Taq DNA Polymerase, 1× SYBR® Green I, 0.1μM ROX reference dye and other optimized buffer components.

Table of Instrument Guide

Instruments	Final Conc. of ROX
ABI PRISM 7000/ PRISM 7700/ 7300/ 7900HT/ StepOne/ StepOnePlus/ GeneAmp 5700	500 nM，high ROX
ABI 7500/ 7500 Fast/ ViiA 7/ QuantStudio 6/7/12K Flex; Agilent Stratagene Mx3000P/ Mx3005P/ Mx4000	50 nM，low ROX
Bio-Rad CFX96/ CFX384/ iQ/ iQ5; MJ Research Opticon 2/ Chromo 4; Roche LightCycler 480/ 96; Corbett Rotor Gene G/ Q/ 3000/ 6000; Thermo PikoReal 96; Eppendorf MasterCycler ep realplex; Cepheid Smart Cycler	No ROX

Protocol

Note: Please follow the procedures outlined in the manual of each respective instrument.

1. Preparation of reaction solution (Take the ABI 7500 as an example)

Add the following reagents to the proper thermal cycler reaction tube or plate on ice:

Component	Volume	Final concentration
2X SYBR® Green qPCR Mix (Low ROX+)	10 μl	1X
Forward Primer (10μM)	0.4 μl	0.2μM
Reverse Primer (10μM)	0.4 μl	0.2μM
Template DNA	variable	0.05-5ng/μl
Water, nuclease-free	to 20 μl	–

Note:

• The primer concentration can be further optimized. The optimal range for primers is 0.1~1μM.
• Prepare according to the recommended volume of each instrument.
• Use 1-10ng cDNA or 10-100ng gDNA for each reaction.
• Users can increase the amount of the the qPCR Mix when using low-copy gene as template.
• Users can reduce the amount of the qPCR Mix, if the melting curve comes with impure peaks.

2. Setup the plate

Transfer the reaction mixture to PCR tubes/plates. Reaction volumes can be reduced to 10 μl if the instrument supports a low volume system.

Cap or seal the reaction tubes/plates then centrifuge briefly to spin down the contents and eliminate any air bubbles.

3. Preform qPCR using the following thermal cycling condition

Set the thermal cycling conditions using default PCR thermal cysling conditions specified in the following tables according to the instrument cycling parameters and melting temperatures of the specific primers.

Standard 3-step PCR mode:

Initial Denaturation	95ºC	20 sec-3 min*	Holding Stage

Denaturation	95ºC	10 sec	Cycling Stage 40 Cycles
Annealing	60ºC	10 sec	
Extension	72ºC	20 sec	
Melting curve analysis			

*20 sec at 95ºC is sufficient time for enzyme activation, however optimal denaturation of complex targets may require up to 3 min denaturation.

Fast 3-step PCR mode (Amplicons 100-150 bp):

Initial Denaturation	95°C	3 min	Holding Stage
Denaturation	95°C	5 sec	Cycling Stage 40 Cycles
Annealing	60°C	5 sec	
Extension	72°C	5 sec	
Melting curve analysis			

Fast 3-step PCR mode (Amplicons 150-300bp):

Initial Denaturation	95°C	3 min	Holding Stage
Denaturation	95°C	5 sec	Cycling Stage 40 Cycles
Annealing	60°C	5 sec	
Extension	72°C	10 sec	
Melting curve analysis			

Note:

SYBR® Green qPCR Mix (Low ROX+) could be used for fast 3-step PCR. And the reaction time could be less than 2-step PCR that using the Mix of other brands.

SYBR® Green qPCR Mix (Low ROX+) contains inhibitors that inhibit the Taq polymerase's activity when under 60ºC, and standard 3-step PCR has better stability and repeatability. So 3-step procedure is recommended rather than 2-step. Customers should confirm that the annealing temperature is above 60ºC when running 2-step PCR.

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 SYBR® 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 20 µl qPCR reaction, use up to 2.0 µl 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 SYBR® 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. 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.

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.