

Cat No. **P2091b** 1mL
P2092b 1mL X 5

2X SYBR® Green qPCR Mix (High ROX)

For research use only

Components

Component	P2091b	P2092b
2X SYBR® Green qPCR Mix (High ROX)	1mL	1mL X 5
Nuclease-free Water	1mL	1mL X 5

Storage

Store at 4 °C for 2 months, protected from light. Store long term at -20 °C, protected from light.

Description

2X SYBR® Green qPCR Mix (High ROX) is designed for high-performance, high-throughput, quantitative Polymerase Chain Reaction (qPCR). The kit contains a *Taq* DNA polymerase developed through protein engineering. The result is a unique enzyme, specifically designed for qPCR using SYBR® Green I dye chemistry.

2X SYBR® Green qPCR Mix (High ROX) is a convenient premix of the components (except primers, template, and water) necessary to perform qPCR using SYBR® Green I dye with enhanced sensitivity and specificity. The SYBR® 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 high ROX reference dye at a final concentration of 500nM.

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 high concentration of ROX reference dye.
- Hot Start technology with anti-*Taq* DNA polymerase antibodies enables high specificity and reproducible amplification.

Composition of the SYBR Green qPCR Mix

100mM KCl, 5mM MgCl₂, 400μM dNTPs, 0.1 U/μL Hot Start *Taq* DNA Polymerase, 1X SYBR® Green, 2% ROX reference dye and other optimized buffer components.

Instrument Guide Table

Instrument	ROX Reference Dye
ABI 5700, 7000, 7300, 7700, 7900HT Step One™ and Step One Plus	High ROX (Cat# P2091b, P2092b) Refer to this instruction manual
ABI 7500 Stratagene Mx3000P®, Mx3005P™, and Mx4000®	Low ROX (Cat# P2091a, P2092a)
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# P2091, P2092)

Protocol

This protocol is intended for use with the ABI 5700, 7000, 7300, 7700, 7900 HT Step One™, and Step One Plus™. This kit is compatible with instruments that need high concentration of ROX Reference Dye (500nM).

1. Preparation of reaction solution

Add all the components to a thin walled PCR tube on ice.

For a total 20μL reaction volume:

Component of reaction mix	Volume	Final concentration
2X SYBR® Green qPCR Mix (High ROX)	10μL	1X
Forward Primer (10 μM)	0.4μL	0.2μM
Reverse Primer(10 μM)	0.4 μL	0.2μM
Template DNA	variable	(<1ng/μL)
Water, nuclease-free	to 20μL	-

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 serial dilution. The concentration is generally <1ng/μL

2. Plate/Tube Setup

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

3. Perform qPCR using the following cycling conditions:

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

*20 sec at 95 °C 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 fluorescence data acquisition at 72 °C.

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 20µL reaction (for smaller volumes, the amount of template should be decreased proportionally). 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 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 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 (T_m) of approximately 60 °C, to take advantage of two-step cycling. If performing quantitative 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 qPCR, melting curve analysis should always be performed to identify

the potential presence of primer-dimers and analyze the specificity of the reaction. Program your thermocycler according to the instructions provided.

SYBR® Green I

2X SYBR® Green qPCR Mix (High ROX) contains an elevated, optimized concentration of the fluorescent dye, SYBR® Green I. High signal intensities are achieved as a result of increased tolerance to high concentrations of SYBR® Green I by the engineered, novel Dongsheng brand SYBR® DNA Polymerase. SYBR® Green I binds all double-stranded DNA molecules, emitting a fluorescent signal on binding. The excitation and emission maxima of SYBR® Green I are at 494 nm and 521 nm, respectively, which are compatible with use on any qPCR instrument.

Magnesium chloride

The MgCl₂ concentration in 2X SYBR® Green qPCR Mix (High 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 qPCR and analyze PCR products in separate areas.
- Set up qPCR mixtures in a laminar flow cabinet equipped with a 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, exodeoxyribonucleases and ribonucleases is confirmed by appropriate quality tests. The product was functionally tested in amplification of a single-copy gene from human genomic DNA.

Product Use Limitations

2X SYBR® Green qPCR Mix (High 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.



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