SYBR® Green qPCR 2x Master Mix

(Cat # 786-1979, 786-1980 & 786-1981)

For research use only

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 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 is specifically designed for qPCR using SYBR® Green I chemistry dye. 2X SYBR® Green qPCR Mix is a convenient premix of the components (except primers and DNA template) that is 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 do not require ROX reference dye
- Hot-start technology brings high specificity and reproducible amplification

Composition of the 2X SYBR Green qPCR Mix

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

Protocol

1. Preparation of reaction solution (Take the Bio-Rad CFX96 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	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 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 cycling conditions specified in the following tables according to the instrument cycling parameters and melting temperatures of the specific primers. A typical PCR cycling program is outlined in the following table.

PCR cycling conditions:			
Steps	Temperature	Time	Cycles
Initial denaturation	95ºC	3 min	1
Denaturation	95ºC	5 sec	40-45
Annealing/Extension	~60ºC	30 sec	
Melting curve analysis	According to instrument guidelines		

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 a 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 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 endo-deoxyribonucleases, exo-deoxyribonucleases 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 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.