Cat. No: P2131, 100 rxns/20-μl rxn
P2132, 500 rxns/20-μl rxn
P2133, 1,000 rxns/20-μl rxn

Super SYBR® Green qPCR Mix

For research use only

Components

Component	P2131	P2132	P2133
2X Super SYBR® Green qPCR Mix	1 ml	1 ml × 5	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

Super SYBR® Green qPCR Mix is a 2X concentrated real-time PCR master mix that requires only the addition of template and primers to perform the reaction. The use of an innovative hotstart mechanism can reduce the interference of primer-dimers and other secondary products on the reaction, which can significantly improve the specificity and amplification efficiency of quantitative PCR, and obtain a wider quantitative amplification region. The new enhancer minimizes fluctuations in PCR efficiency for various target fragments, and repeated freeze-thaw cycles have minimal impact on amplification performance. **This product comes with a special ROX Reference Dye, which is suitable for all qPCR instruments, eliminating the need to adjust the concentration of ROX on different instruments.**

Applications

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

Features

- Compatible with common Real-time systems without the need to adjust ROX reference dye
- Hot-start technology brings high specificity and reproducible amplification

Protocol

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

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

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

Component	10-μl rxn	20-μl rxn	Final Conc.
2X Super SYBR® Green qPCR Mix	5 μl	10 μl	1X
Forward Primer (10μM)	0.2 μl	0.4 μl	0.2μM
Reverse Primer (10μM)	0.2 μl	0.4 μl	0.2μM
Template DNA	0.5-2 μl	1-4 μl	variable
Water, nuclease-free	to 10 μl	to 20 μl	–

Note:

- The primer concentration can be further optimized. The optimal range for primers is 0.2~0.6μM.
- Prepare according to the recommended volume of each instrument.
- Recommended amount of DNA template (10-20 μl system): 1-10ng cDNA or 10-100ng gDNA for each reaction. The amount of cDNA should not exceed 1/10 of the total volume
- Users can increase the amount of the the qPCR Mix to 12 μl when using low-copy gene as template.
- Users can reduce the amount of the qPCR Mix to 8 μl, 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.

2-step PCR mode:

Initial Denaturation	95°C	1 min	Holding Stage
Denaturation	95°C	5 sec	Cycling Stage 40 Cycles
Annealing & Extension	60°C [1]	20~60 sec [2]	
Melting curve analysis (optional) [3]			

3-step PCR mode:

Initial Denaturation	95°C	1 min	Holding Stage
Denaturation	95°C	5 sec	Cycling Stage 40 Cycles
Annealing	55°C [1]	30 sec	
Extension	72°C	30~60 sec [2]	
Melting curve analysis (optional) [3]			

Note:

[1] To improve the specificity, the annealing temperature can be increased. Set up signal collection in the Annealing&Extension or Extension phases respectively.

[2] Consider the instrument type when setting the extension time. If the amplification efficiency needs to be improved, the extension time can be increased.

[3] Different instruments have different melting curve acquisition procedures, generally according to the default

melting curve acquisition procedures of the instrument.

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 60°C. 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

This product 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.