YTA SYBR Green qPCR MasterMix 2X

Cat No :YT2551 **Size: 1 ml** **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

YTA SYBR Green qPCR Mix is considered for high-performance, high-throughput real-time PCR. The kit covers Taq DNA Polymerase engineered through a process of molecular evolution. The result is a unique polymerase that exactly intended for qPCR using SYBR Green I chemistry dye.

YTA SYBR Green qPCR MasterMix is a convenient premix of the components (except primers and DNA template) that essential 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), therefore 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

- This reagent is compatible with many Real-time systems which not require ROX reference dye
- Hot-start technology brings high specificity and reproducible amplification

Composition of the 2× SYBR Green qPCR Mix

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

1. Preparation of reaction solution

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

Component of sample	Volume	Final concentration
2x SYBR Green qPCR Mix	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	variable
Passive reference Dye(50X) optional-not provided	Variable (following table)	1X
Water, nuclease-free	to 20 μl	–

Passive Reference Dye (50X) Guide Table (Cat No : YT2552/1)	
Instrument	Volume
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
ABI Step One,ABI Step One Plus, ABI Prism 7000/7300/7700/7900,Eppendorf	0.4 μl
ABI 7500,ABI 7500 Fast, Stratagene Ms3000 / Mx300SP,Rotorgen 3000	0.08 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.
- 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.perform qPCR using the following 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	95℃	20 sec-3 min*	Holding stage
Denature	95℃	10 sec	Cycling stage 40 Cycles
Annealing	60℃	10 sec	
Extention	72℃	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 150-300 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	10 sec	
Melting curve analysis			

2-step PCR mode

Initial Denaturation	95°C	20sec -3 min*	Holding Stage
Denaturation	95°C	5 sec **	Cycling Stage 40 Cycles
Annealing & Extension	60°C	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.
**Select minimum time (not more than 20 sec) according to instrument user guide.

Note:
SYBR® Green qPCR Mix 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 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 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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