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یکتا تجهیز آزما

YTA Super SYBR Green qPCR MasterMix 2X

For Research Use Only

Components	Cat No:	Size:
2X Super SYBR Green qPCR Mastermix	YT2552	1 ml

Storage
This reagent can be stored at 4°C for 6 months and protected from light. For longer storage, it should be kept at -20°C and protected from light.

Description
2xSuper SYBR Green qPCR mix is designed for high-performance, high-throughput,high-specificity real-time PCR. The kit contains a Hot Start Taq DNA polymerase engineered through a process of molecular evolution. The result is a unique enzyme,specifically designed for qPCR using SYBR® Green I dye chemistry.2xSuper Green qPCR mix is a convenient premix of the components (except primers,template, and water) necessary to perform real-time polymerase chain reaction (PCR)using SYBR® Green I dye with enhanced sensitivity and specificity. The SYBR® Green dyebinds to double-stranded (ds) DNA, thus providing a fluorescent signal that reflects theamount of dsDNA product generated during PCR.

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

Features
• Hot Start technology with updated chemical modification. It gets higher specificity and reproducible amplification.
• Highly reproducible CTs over a broad dynamic range.
• Compatibility with most real-time qPCR instruments.

Composition of the 2x Hy-Super mix
100 mM KCl , 5 mM MgCl2, 400 µM dNTPs, 0.1 U/µl Hot Start Taq DNA Polymerase, 1xSYBR® Green and other optimized buffer components.

Protocol
Note: Please follow the procedures outlined in the manual of each respective instrument.
1. Preparation of reaction solution
Add all the solution in a thin walled PCR tube on ice.

For a total 20µl reaction volume

Component of sample	Volume	Final concentration
2x Super 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	<100ng	
Passive reference Dye(50X) optional-not provided	Variable dye guide table	1X
Water, nuclease-free	to 20 µl	–

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

If the melting curve comes with impure peaks, customer can reduce the 2x Super Green qPCR mix amount in the reaction.

High-Specificity reaction system

Component of sample	Volume	Final concentration
2x Super SYBR Green qPCR Mix	8 µl	1X
Forward Primer (10 µM)	0.4 µl	0.2 µM
Reverse Primer(10 µM)	0.4 µl	0.2 µM
Template DNA	<100ng	
Passive reference Dye(50X) optional-not provided	Variable dye guide table	1X
Water, nuclease-free	to 20 µl	–

If the template is low concentration, customer can increase the 2x Super SYBR Green qPCRmix amount in the reaction.

High-Sensicity reaction system

Component of sample	Volume	Final concentration
2x Super SYBR Green qPCR Mix	12 µl	1X
Forward Primer (10 µM)	0.4 µl	0.2 µM
Reverse Primer(10 µM)	0.4 µl	0.2 µM
Template DNA	<100ng	
Passive reference Dye(50X) optional-not provided	Variable dye guide table	1X
Water, nuclease-free	to 20 µl	–

Note:
1). The primer concentration can be further optimized, if needed. The optimal range for primers is 0.1~1µM.
2). Use 1-10 ng cDNA or 10-100 ng gDNA for each reaction.
3) Prepare in accordance with the recommended volume for each instrument.
4)ROX Reference is required by instruments, for high ROX, add 0.2 µl ROX Reference, and for low ROX, add 0.04 µl ROX Reference.

2. Setup the plate
* Transfer the appropriate volume of reaction mixture to each well of a PCR tube/plate. Reaction volumes may be scaled down from 20 µl to 10 µl if low volume tubes/plates are used.
* Cap or seal the reaction tube/plate and centrifuge briefly.

3. Preform qPCR using the following thermal cycling conditions.

Standard 3 Steps PCR Reaction.

Initial Denaturation	94℃	3 min	Hold
Denature	95℃	10 sec	40 Cycles
Anneal	Tm-5℃	10 sec	
Extend	72℃	20 sec	
Melting curve analysis			

Note: Super SYBR Green qPCR Mix contains reversible inhibitor, it will inhibit the Taq activity below 60℃. So we recommend for 3 step cycling protocols, not 2 steps. If customer use 2 steps PCR Reaction, confirm that anneal and extend temperature should be above 60℃. Numerous tests prove that classical 3 steps PCR Reaction has better stability and repeatability.

Fast 3 Steps PCR Reaction (Amplicons 100-150bp)

Initial Denaturation	94℃	3 min	Hold
Denature	95℃	5 sec	40 Cycles
Anneal	55 ℃	5 sec	
Extend	72℃	5 sec	
Melting curve analysis			

(Amplicons 150-300bp)

Initial Denaturation	94℃	3 min	Hold
Denature	95℃	5 sec	40 Cycles
Anneal	55 ℃	5 sec	
Extend	72℃	10 sec	
Melting curve analysis			

Note: Super SYBR Green qPCR Mix could be used for fast 3 step PCR Reaction. The reaction time could be faster then 2 steps reaction in other brands.

4. Analyze the results
Data analysis varies depending on the instrument used. Please refer to your instrument user guide for information.

Important Notes

Hot Start Technology
Super SYBR Green q PCR Mix uses updated chemical modification hot start technology. It performs excellent specificity and reproducible amplification. Comparing with aitibody hot start, it comes with none animal DNA polluted.

Template
Genomic DNA, plasmid DNA, or cDNA can be used as template. For optimal quantitative results use 20-100ng of genomic DNA or plasmid DNA per 20µl 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.

Primers
Best primers concentration is 0.2-0.6µM. Higher primer concentration gets higher amplification efficiency. But with over amount of primers, it will comes with non-specificy amplification and gets failed results.

3 Steps PCR Reaction
Numerous tests prove that classical 3 steps PCR Reaction gets better stability and repeatability. The optimal temperature of Taq DNA Polymerase is 74℃, most qPCR extend time at 72℃ could be reduced to 5 sec. This could be faster then popular 2 steps PCR Reaction. Initial denaturation for 3 min could active Taq DNA Polymerase completely and also make template DNA departed. This will increase the template sensitivity.