RealAmp™ 2X qPCR Master Mix

Ver 3.0

Low ROX

Cat. No. 801-020 (1.0 ml x 2 tubes)

801-050 (1.0 ml x 5 tubes)

High ROX

Cat. No. 801-021 (1.0 ml x 2 tubes)

801-051 (1.0 ml x 5 tubes)

Storage at -20°C

Disclaimer

For research use only. Not for use in diagnostic or therapeutic procedures.

Description

RealAmp™ 2X qPCR Master Mix is ready to use for Real-Time PCR (qPCR) of Target DNA from DNA or cDNA. This master mix provides fast and robust amplification across a wide range of templates through high quality chemical-mediated hot start PCR enzyme without compromising sensitivity, specificity, dynamic range or PCR efficiency.

RealAmp™ 2X qPCR Master Mix has engineered most optimal reaction buffers to minimize non-specific amplification and/or primer dimer formation. It is a master mix of hot start *Taq* DNA polymerase, Buffer, and dNTP mixture. It has a wide annealing temperature range using chemical-mediated hot start enzyme, so it is possible to specifically amplify a desired target gene.

dsDNA Binding Dye shows higher sensitivity than SYBR Green I or EvaGreen, and has low PCR inhibition, high amplification efficiency and strong fluorescence sensitivity.

Storage Conditions

RealAmp™ 2X qPCR Master Mix can retain the stability of enzyme activity for 1 year at -20°C. To ensure the activity of the master mix, it is strongly recommended to avoid repeated freeze and thaw cycle and to store the mixture in small aliquots. To prevent the excessive exposure of the master mix to the light which leads to loss of fluorescence signal intensity, keep the master mix in dark place. The expiration date is indicated on the label of product box.

Components

Cat. No.	801-020 (200 rxn)	801-050 (500 rxn)
RealAmp™ 2X qPCR Master Mix (Low ROX)	1.0 ml x 2 tubes	1.0 ml x 5 tubes
20X Low ROX reference dye	0.2 ml x 1 tube	0.5 ml x 1 tube

Cat. No.	801-021 (200 rxn)	801-051 (500 rxn)
RealAmp™ 2X qPCR Master Mix (High ROX)	1.0 ml x 2 tubes	1.0 ml x 5 tubes
20X High ROX reference dye	0.2 ml x 1 tube	0.5 ml x 1 tube

PCR Mixture

Components	Volume
RealAmp™ 2X qPCR Master Mix (Low ROX)	10 µl
Forward primer (5 pmol/µl)	1 µl
Reverse primer (5 pmol/µl)	1 µl
Template DNA	- µl
Add D.W. to	20 µl

PCR Conditions

■ 2 Step Cycling Protocol

Step	Temp.	Time	Cycles
Enzyme Activation	95°C	15 min *	1
Denaturation	95°C	20 sec	30~40
Annealing & Extension	55~60°C	30~60 sec	

■ 3 Step Cycling Protocol

Step	Temp.	Time	Cycles
Enzyme Activation	95°C	15 min *	1
Denaturation	95°C	20 sec	30~40
Annealing	55~60°C	30~60 sec	
Extension	72°C	30 sec	

* The chemical-modified HotStart enzyme requires a reactivation at 95°C for 15 min. If sufficient initial denaturation isn't performed, enzyme activity may be inhibited by chemicals that are not completely separated.

References

■ Spectral Characteristics of Fluorescent Dyes

Fluorescent Dyes	Spectral Properties
EvaGreen™	λ abs / λ em = 500/530 nm (with DNA) λ abs = 471 nm (without DNA)
SYBR Green I	λ abs / λ em = 497/520 nm (with DNA)
dsDNA Binding Dye	λ abs / λ em = 459/522 nm (with DNA)
FAM	λ abs / λ em = 494/520 nm (with DNA)

■ PCR Cycle According to Sample Type & Concentration

Sample	Concentration of Template		
	1~5 ng	10~50 ng	50~200 ng
Animal genomic DNA	-	30~50 cycles	30~40 cycles
Bacterial genomic DNA	35~50 cycles	30~40 cycles	-
Plasmid DNA & λ DNA	30~40 cycles	-	-

* If the concentration of template DNA is too high, background fluorescence may increase and it may interfere with the formation of a stable amplification curve (200ng or less recommended).

** In the case of cDNA Template for RT qPCR, cDNA stock solution (or 1/10 diluted cDNA) synthesized from 1ug Total RNA is used, and the amount added should not exceed 10% of the final qPCR volume.
(e.g., 2 µl of cDNA Template is used when total qPCR reaction volume is 20 µl)

■ ROX Final Concentration for Different Instrument

The ROX reference dye is inert, which means that it does not undergo any fluorescence change during real-time quantitative PCR. Therefore, the addition of a ROX reference dye helps normalize the fluorescent reporter signal by allowing the software/instrument to adjust for minute differences or well-to-well inconsistencies. In this way, a ROX reference dye enables minimal standard error and improves the overall performance of each experiment. Every company provides the guidance for using ROX reference dye on their instrument. RealAmp™ 2X qPCR Master Mix comes with a ROX reference dye to suit their requirement. Use a ROX reference dye according to your instrument in the tables below.

ROX		Real-time PCR Instrument
No ROX	Bio-Rad	iCycler™
		MyiQ™
		MiQ™ 2
		iQ™ 5
		CFX-96 Touch™
		CFX-384 Touch™
		CFX Connect™ Real-time PCR System
		Chromo4™
		Opticon™
		Opticon™ 2
		MiniOpticon™
	QIAGEN	Rotor-Gene® Q
		Rotor-Gene® 3000
		Rotor-Gene® 6000
	Eppendorf	Mastercycler® ep realplex
	illumina	Eco™ Real-time PCR System
	Cepheid	SmartCycler®
	TaKaRa	Thermal Cycler Dice™ Real-time System

ROX		Real-time PCR Instrument
Low ROX	ABI	7500
		7500 Fast
		7500 Fast Dx
		ViiA 7™
		QuantStudio™ 3
		QuantStudio™ 5
		QuantStudio™ 6
		QuantStudio™ 7
		QuantStudio™ 12K Flex
	Stratagene	Mx3000P
		Mx3005P
		Mx4000P

ROX		Real-time PCR Instrument
High ROX	ABI	7500
		7000
		7300
		7700
		7900
		7900HT
		7900HT Fast
		StepOne™
		StepOnePlus™
	Stratagene	Mx3000P
		Mx3005P
		Mx4000P

Trouble Shooting Guide

Facts	Possible Causes	Suggestions
No or weak amplification	Too short annealing or extension time	Keep the recommended time for annealing and extension. In some cases, it can be helpful to expand the time up to 10 sec.
	Too old or mis-stored primers	Use fresh primers if possible. Primers can be damaged gradually even at -20°C. Repeated freezing and thawing will accelerate the damage. Storing the primers in small aliquots can be helpful retard its damage.
	No or damaged template in the sample	Validate the method for sample preparation or the sample itself by proper analysis method. The sample prepared by inappropriate method can contain heavily damaged or no nucleic acid.
	The concentration of the mixed component is out of valid range.	This can be caused by imprecise pipette or user's mishandling. Calibrate the pipettes periodically and follow the instructions of the provider for the maintenance.
	Too long target for qPCR	The optimal amplicon size is between 80-150 bp. If the length of target is larger than 300 bp in length, the efficiency of PCR reaction will be significantly decreased.
	Wrong storage of RealAmp™ 2X qPCR Master Mix	Avoid exposure to light which can reduce the sensitivity of dsDNA Binding Dye. Store the RealAmp™ 2X qPCR Master Mix in small aliquots at -20°C for the preservation of proper activity.
Interferential effect on the amplified signal	Incorrect use of ROX reference dye	Use of too much ROX can lead to normalized but lower signal than the expected, while too little ROX may cause a less-normalized signal. Refer to the 'ROX Final Concentration for Different Instrument' for the correct use of ROX.
Amplified in negative control	Mixture contamination	Contamination can be accidently happened during the preparation of mixture. Be sure to clean the circumstances and to use sterile consumables.
	Formation of primer dimers	The formation of primer dimers interfere with the analysis by real-time PCR when it comes to the cause of higher background. Optimized the concentration or the design of primers.