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Datasheet

Ver.1 Date : 20180222

2X ACE SYBR® Color qPCR Master Mix

Cat# EP2019 – 1.25 ml / EP2020- 5 ml / EP2021 - 25ml

Storage at -20 ℃ for one year

INTRODUCTION

The **2X ACE SYBR® Color qPCR Master Mix**, protected by Taq DNA Polymerase via an antibody-modified hot-start activation technique, is specially designed for SYBR Green I based quantitative PCR (qPCR). Unique factors in the optimized buffer system of **2X ACE SYBR® Color qPCR** Master Mix significantly improve its sensitivity and specificity. The mix is prepared at 2x reaction concentration and can be directly used for robust and low-template qPCR with high sensitivity, specificity, and reliability. Furthermore, the **2X ACE SYBR® Color qPCR Master Mix** contains tracking dyes to reduce pipetting errors.

CONTENTS

No	Component	EP2019-1.25 ml	EP2020-5 ml	EP2021-25 ml
DA	2X ACE SYBR® qPCR Master Mix[1]	1.25 ml	1.25 ml x 4	1.25 ml x 20
DB	10X Dilution Buffer[2]	1.25 ml	1.25 ml x 4	1.25 ml x 20
DC	50X ROX Reference Dye1[3]	50 ul	200 ul	200 ul x 5
DD	50X ROX Reference Dye2[3]	50 ul	200 ul	200 ul x 5

1. Contains dNTPs, Mg^{2+}, Hot-Start Taq DNA Polymerase, SYBR Green I, etc.

2. Contains Yellow tracking dye, and is used for the dilution of templates.

3. Used to rectify the error of fluorescence signals between different wells. Select the appropriate ROX reference dye according to the Real-time PCR instrument used:

DO NOT USE ROX Reference Dye	Bio-Rad CFX96™, CFX384™, iCycler iQ™, iQ™5, MyiQ™, MiniOpticon™, Opticon®, Opticon 2, Chromo4™; Cepheid SmartCycler®; Eppendorf Mastercycler® ep realplex, realplex 2 s; Illumina Eco qPCR; Qiagen/Corbett Rotor-Gene® Q, Rotor-Gene® 3000, Rotor-Gene® 6000; Roche Applied Science LightCycler™ 480; Thermo Scientific PikoReal Cycler.
USE ROX Reference Dye 1	Applied Biosystems 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast; StepOne™, StepOne Plus™.
USE ROX Reference Dye 2	Applied Biosystems 7500, 7500 Fast, ViiA™7; Stratagene MX4000™, MX3005P™, MX3000P™.

PROTOCOL

1. Dilution of Templates

The **2X ACE SYBR® Color qPCR Master Mix** contains Blue dye, and the 10x Dilution Buffer contains Yellow dye. The templates are dissolved with 10x Dilution Buffer (Yellow) and are then pipetted to 2X ACE SYBR® Color qPCR Master Mix (Blue). The mixture will turn Green immediately. Based on this color change, the user can easily determine whether the template is added or not. Please use the 10x Dilution Buffer to dilute the templates as follows. However, if no tracking is needed, the 10x Dilution Buffer will not be necessary.

Template Types	Examples	Usage of 10× Dilution Buffer	Concentration of 10× Dilution Buffer in Template
Plaque or Powder	Plaque of undissolved DNA precipitation	Dilute the 10 x Dilution Buffer with ddH_2O to 1×, then dissolve the template with 1× Dilution Buffer.	1 x
Solution	cDNA solution Dissolved plasmids/gDNA	If necessary, dilute the template with ddH_2O to an appropriate concentration, then add 1 µl of 10x Dilution Buffer to 9 µl of diluted template.	1 x

NOTE :

1. Other approaches are also applicable. Please ensure the final concentration of Dilution Buffer in template solution is 1×.
2. In a qPCR reaction system of 20 µl, the volume of the template (in 1x Dilution Buffer) should be within the range of 2 µl-5 µl. A template volume of <2 µl/20 µl leads to a lighter color, which may not be distinguishable during color changes. While a template volume of >5 µl/20 µl will disturb the qPCR reaction.
3. The 1× Dilution Buffer, with a final concentration of ≤ 5 µl/20 µl reaction system, is safe and not harmful for qPCR reactions.
4. A white baseboard (provided with this kit) is recommended for a better observation of color changes.

2. Mix the following components

White precipitation may appear during the thawing of the APOLO SYBR® Color qPCR Master Mix. Before use, dissolve the precipitation by incubating at room temperature and gently flipping the tube. Mix the solution thoroughly every time before pipetting.

2X ACE SYBR® qPCR Master Mix	10 ul
Primer 1 (10 uM)	0.4 ul
Primer 2 (10 uM)	0.4 ul
Template DNA / cDNA	
50X ROX Reference Dye	0.4 ul
RNase-free ddH_2O	To 20 ul

For each component, the volume of can be adjusted according to the following principle:

a. The final concentration of primers usually 0.2 µM, and if necessary, it can be adjusted between 0.1 µM and 1.0 µM.

b. The accuracy of template volumes impacts significantly on the qPCR results, due to the high sensitivity of APOLO SYBR® Color qPCR Master Mix. It is recommended to add 2 µl-5 µl of template (in 1× Dilution Buffer) to the 20 µl reaction. Especially, for undiluted cDNA, the volume of the template should be ≤ 1/10 of total volume.

c. The size of the amplicon should be within the range of 80-150 bp.

3. Place the sample in a qPCR instrument and run the following program for qPCR:

Stage	Temp.	Time	Cycle
Pre-Denaturation[1]	95℃	30 s	1
Denaturation	95℃	10 s	40
Annealing + Extension[2]	60℃	30 s	
Melting Curve[3]	95℃	15 s	1
	60℃	60 s	
	95℃	15 s	

1. Pre-denaturation at 95℃ for 30 sec is suitable for most amplification. However, it could be prolonged to 3 min for templates with complicated structures.
2. Extension for 30 sec is suitable for amplicons ≤ 300 bp. It is recommended to prolong extension to 60 sec for amplicon > 300 bp.
3. Program for melting curve may vary qPCR instruments. Please select the default melting curve program of the instrument used.

OPTIMZING REACTION SYSTEM

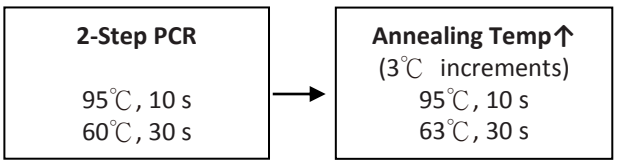
Features of a good qPCR system include (list in order of importance) **a single peak in melting curve** (indicating high amplification sensitivity), an **e value close to 100%** (indicating high amplification efficiency), and a **low Ct value** (indicating high amplification efficiency). If failed to get good qPCR performance using the default qPCR program, optimize the reaction system to improve the amplification sensitivity and efficiency according to the following guidelines:

1. The relationship between primer concentration and qPCR performance: when the final concentration of primer ranges from 0.1 µM to 1.0 µM, increasing the primer concentration will lead to decrease in amplification specificity and improvement in amplification efficiency.

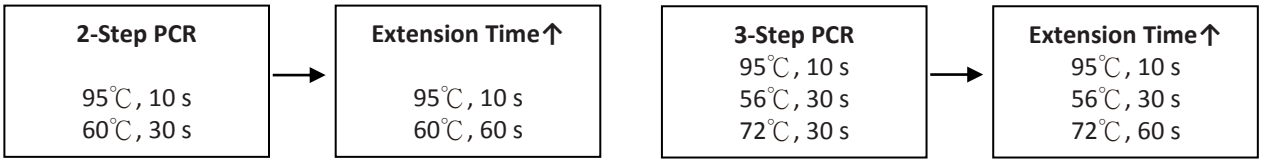
2. Pre-denaturation time: Pre-denaturation at 95℃ for 30 sec is suitable for most templates. Extend the pre-denaturation time to 3 min for template DNA with complicated structures.

3. The relationship between qPCR program and performance:

To improve specificity, select a 2-step PCR program or increase the annealing temperature.



To improve efficiency, increase extension time or switch to a 3-step PCR program.



4. Primer Design Notes

1. The amplicon size should be 80 bp-150 bp.

2. The primer length should be 17 bp-25 bp.

3. Avoid GC-rich and AT-rich region at the 3'-end of the primer.

4. Choose C or G, instead of T, as the last base of the 3'-end of the primer.

5. The difference in Tm value between the forward and reverse primer should be ≤ 1℃. Tm values of primers should be with 60℃-65℃ (calculated with Primer 5).

6. GC content of the primers should be within the range of 40%-60% or 45%-55% as preferred.

7. A, G, C and T should be distributed as equally as within the primer. Avoid using GC- or TA-rich regions.

8. Avoid (self-) matching of ≥ 8 bases between all primers. At the 3'-ends, avoid matching of 3 bases between the forward and reverse primers.

9. Analyze the primers using the BLAST program on NCBI to eliminate the possibility of non-specific amplification.

PRODUCT USE LIMITATION

These products are intended for research use only.