

SYBR® Green Real Time PCR Master Mix

Description:

2X SYBR® Green Real Time PCR Master Mix is a very sensitive and easy to use for real-time quantitative analysis of DNA and cDNA targets from various sources. This product is based on the SYBR Green I and a dual Hot-start *Taq* DNA polymerase (chemically modified and anti *taq*) plus the pre-optimized buffer solution.

Buffers and Reagents:

Storage Buffer: 20 mM Tris-HCl (pH 8.0), 100 mM KCl, 0.5 mM EDTA, 0.1 mM DTT, 0.5% Tween 20, 0.5% Nonidet P-40, 50% Glycerol
2X Master Mix (per 10 µL): 1unit Hot start *Taq* DNA Polymerase, 5 mM MgCl₂, 2mM dNTPs mixture and 2X SYBR Green I

Kit contents

Component	Volume
SYBR® Green PCR Master Mix (2X)	1000 µL
50X ROX dye	50 µL

Kit storage:

This kit should be stored at -20°C. Unnecessary repeated freeze/thawing should be avoided..

For frequent use, SYBR® Green Real Time PCR Master Mix can be stored at 2-8 C for 2 months.

If the Reagents have been thawed but not used, it is important to thoroughly mix prior to re-freezing. (The layering of salts during the thawing process and subsequent crystallization during freezing will damage the enzyme and decrease product performance).

General Reaction Protocol:

1. Thaw 2X SYBR® Green Real Time PCR Master Mix.

Component	Volume	Final conc.
2X Master Mix	10 µL	1X
50X ROX dye (If needed)	0.4 µL	1X
Forward Primer (10 pmoles/µL)	0.2~2.0 µL	0.1~1.0 pmoles
Reverse Primer (10 pmoles/µL)	0.2~2.0 µL	0.1~1.0 pmoles
Template DNA	Variable	10 fg~1 µg
Sterilized D.W.	Variable	-
Total Volume	20 µL	-

2. Prepare a master mix. Gently mix reagents by inverting the tube and centrifuge. DO NOT vortex and avoid producing bubble.

3. Mix the master mix thoroughly and dispense appropriate volumes into PCR tubes or plates.

4. Add templates DNA to the individual PCR tubes or wells containing the master mix.

5. Program the Real-Time PCR machine according to the program outlined.

6. Place the PCR tubes or PCR plates in the terminal cyclor and start the cycling program.

7. Perform a melting curve analysis of the PCR product(s).

Cycle	Time	Temp °C
1	15 Min*	95
	15 Sec*	95
35 ~40	30 Sec	55~65
	30 Sec	72

Melting/Dissociation Curve Stage

*This item should be set as mentioned in our [protocol](#).