2X qPCR Master Mix with ROX (SYBR)

(Cat. No.: S2GNM02j30001)

Description:

2X qPCR Master Mix with ROX (SYBR) is a convenient and complete real-time quantitative PCR (qPCR) reagent containing hot-start *Taq* polymerase in an optimized buffer and SYBR green fluorescent dye to ensure accurate and sensitive amplification. The real-time tracking of the amplification process and simultaneous quantification of targeted DNA molecules are also enabled. The ROX reference dye in master Mix facilitating normalization of each qPCR assay. Additionally, the inclusion of an inert smart blue contrast dye ensures the 2X qPCR Master Mix with ROX (SYBR) is ready-to-use, reducing pipetting errors, and greatly improving the reproducibility of the process.

Kit Contents:

Contents	S2GNM02j30001 (100 rxns)
2X qPCR Master Mix with ROX (SYBR)	1 ml

Storage:

This product is stable for 12 months at -20°C.
It is suggested to keep protected from light and aliquot to avoid multiple freeze-thaw cycles.

Protocol:

1. Thaw 2X qPCR Master Mix with ROX (SYBR) at 4°C. Mix thoroughly and centrifuge briefly, then keep at 4°C and avoid from light.
2. Prepare the reaction mix according to the recommendations in Table 1. Vortex thoroughly and centrifuge briefly.

Table 1. Reaction Setup

Components	Volume per 20µl reaction	Final Concentration
2X qPCR Master Mix with ROX (SYBR)	10 µl	1X
Forward primer	Variable	50 – 400 nM
Reverse primer	Variable	50 – 400 nM
DNA template	2 µl	cDNA: 100fg–100ng

		Genomic DNA: 80pg-50ng Plasmid: 10^2-10^8 molecules
Nuclease-free H_2O	to a final volume of 20µl	-

Note: PCR primer's concentrations for optimal qPCR reactions may vary depending on primer properties and template condition.

3. Setup the thermal cycling program on a real-time PCR system as described below (Table 2 or 3).
4. Load the PCR tubes or plate into the real-time PCR instrument and start the program.

Recommended real-time PCR Program

Table 2. Two-step Cycle

Step	Temperature	Time	Cycles
Template denaturation and enzyme activation	95°C	10 mins#	1
Denaturation	95°C	15 sec	40
Annealing and Extension	60°C	60 sec	
Melting curve analysis	Refer to the instrument manual		

Table 3. Three-step Cycle

Step	Temperature	Time	Cycles
Template denaturation and enzyme activation	95°C	10 mins#	1
Denaturation	95°C	15 sec	40
Annealing	55-60°C	30 sec	
Extension	72°C	30 sec	
Melting curve analysis	Refer to the instrument manual		

10 minutes is recommended for the first step to completely denature the DNA and activate the enzyme.

◎ **Revision History** ◎

Description	Version	Date
Initial Release	S2GNM02j30001_Protocol_V1	Aug 2023