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Advanced Sybr GREEN® qPCR Super Mix (Low ROX & High ROX)

Product Description

Advanced Sybr qPCR SuperMix is a 2X Ready-to-Use Hot-start mix containing Hot Start DNA polymerase, dNTPs, $MgCl_2$, ROX, Sybr Green dye, except primers & templates for real time quantitative PCR.

The Mixture has been optimized for excellent performance and provide higher processivity.

Advanced Sybr Green® qPCR Super Mix (LOW ROX)

Description	Cat #	Size
Advanced Sybr Green qPCR Super Mix (Low ROX)	**FS-T-71345-5**	**500 rxn (20 ul) (5 X 1 mL)**
Advanced Sybr Green qPCR Super Mix (Low ROX)	**FS-T-71345-20**	**2000 rxn (20 ul) (2 X 10 mL)**

Advanced Sybr Green® qPCR Super Mix (HIGH ROX)

Description	Cat #	Size
Advanced Sybr Green qPCR Super Mix (High ROX)	**FS-T-71346-5**	**500 rxn (20 ul) (5 X 1 mL)**
Advanced Sybr Green qPCR Super Mix (High ROX)	**FS-T-71346-20**	**2000 rxn (20 ul) (2 X 10 mL)**

Sybr Green® dye binds directly to dsDNA generated during amplification, which permits saturation dye concentration in qPCR without PCR inhibition this dye is ideal for both qPCR and HRM High Resolution Melting analysis

The absorption and fluorescence emission spectra of DNA-bound Sybr Green® Dye are very similar to those of FAM with Ex/Em at 500/530 nm with DNA.

Advanced Sybr Green Super Mix contains HotStart Taq DNA Polymerase chemically modified that is completely inactive at RT. Upon Heat Activation Hot Taq is fully activated in 2 minutes fast cycling PCR, this enables specific and efficient primer extension.

• Gel electrophoresis analysis of PCR products: After PCR with Sybr Green® Dye, PCR products need not be stained with another DNA gel stain. Simply add DNA loading buffer to your PCR reaction solution, load on a gel, and conduct electrophoresis as usual. Gel visualization can be carried out using a 254 nm UV box, or a blue LED imager using a SYBR® Green filter. Alternatively, the gel may be imaged using a 488 nm laser-based gel scanner.

REACTION SET UP

Thaw all components at RT, mix thoroughly by inverting the tube/bottle several times to ensure homogeneity as a concentration gradient may form during -20°C storage. Centrifuge to collect contents at the bottom of the tube

Reagents	10ul reaction	20ul reaction	Final conc.
Advanced Sybr Green qPCR 2x Super Mix (Low ROX or High ROX)	5 ul	10 ul	1X
10 um Forward Primer*	Variable	Variable	0.1~0.5 uM
10 um Reverse Primer*	variable	variable	0.1~0.5 uM
Template**	50 pg to 50 ng	50 pg to 50 ng	NA
Water RNase Free	Up to 10ul	Up to 20ul	

General Considerations

- * Primer design and amplicon length: For optimal results, use appropriate software to design primers with melting temperatures (Tm) of approximately 60°C that amplify products of 60-200 bp. For longer amplicons, extension times may need to be extended.

** **Template concentration:** We recommend 50 pg to 50 ng genomic DNA per reaction. The optimal amount of template DNA varies by application.

- Sybr Green® Dye can be used for high resolution melting (HRM) analysis. Follow the qPCR system's instructions for data collection and analysis.

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Cycling Protocol

Choice of cycling protocol depends on your instrument capability and on the nature of your amplicon. If your instrument does not support fast cycling, use the parameters recommended in your instrument manual.

A. Two-step fast cycling protocol

This cycling protocol should be applicable to most amplifications where the Melting temperature of the primers is designed to be 60℃.

Cycling Step	Temp	Holding Time	Number of cycles
Enzyme activation	95℃	2 min	1
Denaturation	95℃	2-5 sec	40
Annealing / extension / data acquisition	60℃	20-30 sec	
Dissociation / melt curve	Set up as per instrument guidelines		

B. Three-step fast cycling protocol

Use this protocol when optimal primer annealing and extension temperatures are desired.

Cycling Step	Temp	Holding Time	Number of cycles
Enzyme activation	95℃	2 min	1
Denaturation	95°C	2-5 sec	40
Annealing	55-65°C	10 sec	
Extension / data acquisition	72°C	10-20 sec	
Dissociation / melt curve	Set up as per instrument guidelines		

- For two-step RT-PCR: the A_{260} measurement of a reverse transcription reaction does not accurately quantify cDNA. Add undiluted or diluted cDNA from a RT reaction (generated from < 1 ug RNA), but the RT reaction volume must not exceed 10% of the final PCR volume.

Table 1. Instrument Compatibility

• For certain instruments, ROX is necessary for accurate Ct determination from well to well. Refer to **Table 1** for the recommended ROX concentration (high or low) for your instrument. ROX may add noise to melt curve analysis, which could be mistaken for real peaks. Thus, in case of unexpected melt-curve peaks, un-check “ROX” in the “Passive Reference Dye” box in the software so that data is not collected from the ROX fluorescence channel, then re-analyze the data.

- Roche LightCycler® users: If using glass capillaries for reactions, add BSA to the PCR reactions at ~250 ng/uL final concentration. BSA is not necessary if plastic capillary tubes are used.

PCR Instrument	Recommended ROX Concentration	Amount of ROX per 20 uL reaction
BioRad: iCycler™, MyiQ™, MiQ™ 2, iQ™ 5, CFX Opus, CFX-96 Touch™, CFX-384 Touch™ and Connect™, Chromo4™, MiniOpticon™ Qiagen: Rotor-Gene® Q, Rotor-Gene® 3000 & 6000 Eppendorf: Mastercycler® Realplex Illumina: Eco™ RealTime PCR System Cepheid: SmartCycler Roche: LightCycler® 480, LightCycler® 2.0	No ROX	None Required **Note:** Bio-Rad’s iCycler™, MyiQ™, MiQ™, and iQ™ users do not need to add fluorescein to the PCR reaction as Sybr Green® dye has a slight background fluorescence that provides adequate and stable baseline level fluorescence.
Applied Biosystems®: 7500, 7500 Fast, ViiA™7, QuantStudio® instruments Stratagene (Agilent): MX4000P, MX3000P, MX3005P	Low ROX (~50 nm)	If using Template Buffer, dilute ROX 1/10 with dH_2O and add 1.8 uL diluted ROX per 20 uL reaction. Or add 18 uL undiluted ROX per 1 mL tube of master mix.
		If not using Template Buffer, dilute ROX 1/100 with dH_2O and add 3 uL diluted ROX per 20 uL reaction. Or add 3 uL undiluted ROX per 1 mL tube of master mix
Applied Biosystems®: 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™, StepOnePlus™	High ROX (~500 nm)	If using Template Buffer add 2 uL ROX Reference Dye per 20 uL reaction.
		If not using Template Buffer, dilute ROX 1/10 with dH_2O and add 3 uL diluted ROX per 20 uL reaction. Or add 30 uL undiluted ROX per 1 mL tube of master mix.
BioRad: iCycler™, MyiQ™, MiQ™ 2, iQ™ 5	Fluorescein*	None Required

*Bio-Rad’s iCycler™, MyiQ™, MiQ™, and iQ™ users do not need to add fluorescein to the PCR reaction as Sybr Green® Dye has a slight background fluorescence that provides adequate and stable baseline level fluorescence. For these instruments we recommend using Sybr Green® qPCR Master Mix without ROX.