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2X FAST EVA GREEN qPCR SUPER MIX FS-T-41310

Description

Fast EvaGreen qPCR SuperMix is a 2X Hot Start mix containing dNTPs, HotStart Taq,$MgCl_2$, EvaGreen dye.
The Master Mix was formulated to for Fast Cycling PCR, and can be used for regular cycling protocols.
The optional **40x template buffer** helps to track where DNA templates have been added to the reaction mixes. It offers high sensitivity, robust fluorescence signal amplification.
EvaGreen® 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 EvaGreen® Dye are very similar to those of SYBR® Green I or FAM with Ex/Em at 500/530 nm with DNA.

Kit Components

Cat.n.	Fast Eva Green qPCR Super Mix	Size
FS-T-41310-5	2X Fast Eva Green qPCR Super Mix (No ROX)	5 x 1 ml
	40x Template Buffer	2 x 1 ml
FS-T-41315-5	2X Fast Eva Green qPCR Super Mix (Low ROX)	5 x 1 ml
FS-T-41316-5	2X Fast Eva Green qPCR Super Mix (High ROX)	5 x 1 ml

1 ml = 100 reactions (20µl volume)

Additional Material Required but not Supplied

1. Optical-grade qPCR tubes, plates, sealing films, and aerosol-resistant pipette tips
2. qPCR primers and probes DNA or cDNA templates

Applications

- Real-time PCR
- Gene expression profiling
- Gene knockdown verification
- Array validation

Note

Do not contaminate 2X Fast EVA Green qPCR Super Mix with primers, probe and template DNA used in individual reactions. Thaw and mix all components thoroughly, spin down shortly and chill on ice.

Precautions

1) Ensure that the reagent is fully thawed before use. Gently mix before use to avoid creating bubbles, then thoroughly and centrifuge before use. After use, immediately return it to -20°C

2) This product contains polymerase. During use, keep it on ice. If multiple uses are required within a short period, it can be temporarily stored at 4°C. Repeated freeze-thaw cycles should be avoided as much as possible.

3) Choose an appropriate reference dye based on the qPCR machine model you are using.

4) When preparing and dispensing the reaction mixture, use sterile pipette tips, preferably those with filters.

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.
• Gel electrophoresis analysis of PCR products: After PCR with EVA 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.

Protocol

1- The following table shows recommended component volumes:

Reaction Conditions

Reagents	10 µl	20 µl	Final Conc.
2x Fast EVA Green qPCR Super Mix	5 µl	10 µl	1X
40x Template Buffer (optional)***	(optional)	(optional)	
ROX Dye (10X)	(optional)	(optional)	
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 10µl	Up to 20µl	

***Please note "Use of the ROX Reference Dye only if required**

***The use of Template Buffer** is optional, but all reactions in a given experiment should contain the same amount for accurate comparisons. Template Buffer should be at 1X in the final reaction. If 1 uL of DNA is to be added to each 20 uL reaction, mix 40X Template Buffer with DNA at a ratio of 0.5 uL Template Buffer per 1 uL DNA, then add 1.5 uL of the mix to each reaction. If 5 uL of DNA is to be added to each reaction, mix at a ratio of 0.5 uL 40X Template Buffer per 5 uL DNA, and then add 5.5 uL of the mix per reaction. When using small volumes of template it may be convenient to dilute 40X Template Buffer with PCR grade water prior to use. For example, you could mix 1 uL of 20X Template Buffer per 1 uL DNA, then add 2 uL of the mix to each reaction.
Note: **Template Buffer quenches ROX fluorescence**. Refer to Table 1 for the recommended ROX concentrations when Template Buffer is used.

Template concentration: The optimal amount of template DNA varies by application. We recommend 50 pg to 50 ng genomic DNA per reaction. For two-step RT-PCR: the A260 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.

Cycling protocols

Depends on your instrument.

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°C	2-5 sec	40
Annealing	55-65°C	10 sec	
Extension / data acquisition	72°C	10-20 sec	

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	

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.

2.

PCR Instrument	ROX Conc.	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 EvaGreen® 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 EvaGreen® Dye has a slight background fluorescence that provides adequate and stable baseline level fluorescence. For these instruments we recommend using EvaGreen® qPCR Master Mix without ROX .

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