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EVA GREEN qPCR 2X Master MIX FS-T-1271

Description:

Eva Green qPCR 2X Master Mix is a ready-to-use hot-start mix for qPCR and DNA melt curve analysis of PCR amplicons. It is formulated for qPCR using a fast-cycling protocol, but also can be used for qPCR with regular cycling protocols. Eva Green dye is a unique DNA-binding dye with features ideal for both qPCR and melt curve analysis. Green dye binds to dsDNA via a novel "release-on-demand" mechanism, which permits the use of a relatively high dye concentration in qPCR without PCR inhibition.

Eva Green qPCR 2X Master Mix contains our proprietary chemically-modified Hot-Start Taq DNA Polymerase. Unlike AmpliTaq Gold, which is also a chemically modifiedTaq but takes 10 minutes or longer to activate, this Taq DNA Polymerase is fully activated in 2 minutes with high activity recovery, making it particularly suitable for fast PCR. HS-Taq is completely inactive at room temperature and largely free of DNA contamination. This makes HS-Taq superior to any antibody-based hot-start Taq, which istypically not completely inactive at room temperature and is prone to DNA contamination due to the nature of antibody production. The Eva Green dye in the Master Mix can act as a DNA pre stain, permitting direct visualization of DNA bands following electrophoresis.

Kit Contents

Contents	FS-T-1271
Eva Green qPCR 2X Master Mix	1 ml*
10x Rox Dye	1 ml

***1 ml = 100 reactions**

The product contains two components.

Component A contains EVA Green dye, dNTP, PCR buffer (including Tris and MgCl2) and hot-start Taq polymerase.

Component B is 10X Rox reference, which may be required on certain ABI instruments (See protocol below).

Spectral Properties : λabs/λem = 500/530 nm (bound DNA) λabs = 471 nm (without DNA)

Applications

- **Real-time PCR**
- **Detection and quantification of DNA and cDNA targets**
- **Gene expression profiling/Gene knockdown verification**
- **Microbial detection**
- **Viral load determination**
- **Array validation**
- **SNP genotyping**
- **HRM**

Protocol

1. Thaw reagents at room temperature. Mix thoroughly and then place on ice immediately after thawing.
2. Assemble reaction tubes on ice whenever possible to avoid premature, non specific polymerase activity.
3. The following table shows recommended component volumes:

Reaction Conditions PCR

Reagents	20ul reaction	Final conc.
Eva Green qPCR 2x Master Mix	10.ul	1X
ROX Dye (10X)	*(optional)	See note 4
10 um Forward Primer	X ul each	0.1~0.5 uM
10 um Reverse Primer	X ul each	0.1~0.5 uM
Template**	Variable (see note 1 and 2)	NA
Water RNase Free	Up to 20ul	

Notes

cDNA templates: EVA Green qPCR 2X Master Mix is suitable for mRNA quantitation if a two-step procedure is followed. The first step involves converting the mRNA to cDNA by reverse transcription (components not provided). A portion of the synthesized cDNA can then be quantitated by using EVA Green qPCR 2X Master Mix in the second step. To ensure optimal amplification efficiency, the aliquot of the cDNA sample to be amplified should not exceed 10% of the volume of the PCR reaction. We recommend cDNA synthesis kits For accurate quantitation of transcript levels, a no-RT control is recommended to check for possible genomic DNA contamination.

1. **One-step RT-qPCR** can also be applied for mRNA quantitation. Primer sets must be well characterized to ensure no primer-dimer formation. We recommend that you titrate the amount of reverse transcriptase and the duration of the RT step. If possible, design primers to have Tm at 55 ℃, run both RT step and extension step at 55 ℃. For accurate quantitation of transcript levels, a no-RT control is recommended to check for possible genomic DNA contamination.
2. **Template concentration**: The optimal amount of template DNA varies by application. Recommended amounts of genomic DNA template per reaction typically range from 50 pg to 50 ng per reaction. Recommended amounts of cDNA typically range from 50 fg to 50 pg, based on the amount of input RNA in the RT reaction.
3. **ROX reference dye**: For certain instruments, ROX is necessary for accurate Ct determination from well to well. Refer to Table 1 (See Page 5-6) for therecommended ROX concentration for your instrument. ROX may add noise to melt curve analysis, which could be mistaken for real peaks. Thus, in case of unexpected peaks, uncheck "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.

Cycling Protocols

You may choose one of the following three protocols, depending on the nature of your amplicon and instrument capability.

A. Universal cycling protocol

This cycling protocol can be used on nearly all qPCR instruments. The protocol also may be useful for targets that are relatively difficult to amplify underfast cycling conditions.

Cycling Step	Temp.	Holding Time	N.of Cycles
Enzyme Activation	95 ℃	2 min	1
Denaturation Annealing & Extension	95 ℃ 60 ℃	15s 60s	45

B. Two-step fast cycling protocol

This cycling protocol should be applicable to most amplifications where the primer Tm's are designed to be 60℃. Melt curves may be performed by following instructions provided for your instrument.

Cycling Step	Temp.	Holding Time	N.of Cycles
Enzyme Activation	95 ℃	2 min	1
Denaturation Annealing & Extension	95 ℃ 60 ℃	5s (Note 5) 30s	45

C . Three-step fast cycling protocol

This cycling protocol can be used if you would like to have the extension step to be performed at a higher temperature than the annealing step. For example, if you have relatively long primers that tend to anneal non-specifically, carrying out the extension step at a higher temperature can reduce nonspecific amplification. Melt curves may be performed by following instructions provided for your instrument.

Cycling Step	Temp.	Holding Time	N.of Cycles
Enzyme Activation	95°C	2 min	1
Denaturation Annealing Extension	95 ℃ 50~60℃ 72 ℃	10 s 10s Note 6) 10s (Note 7)	45

General Considerations

1.qPCR instruments: For iCycler users, you do not need to add FAM to your PCR mix as Eva Green dye has a slight background fluorescence that provides adequate and stable baseline level fluorescence. For Roche LightCycler users using glass capillaries for reactions, you need to add BSA to your PCR reactions (~0.5 mg/mL final concentration). BSA is not necessary if transparent plastic capillary tubes are used.

2. Instruments for melt curve analysis: Suitable instruments include Rotor-Gene 6000, ABI 7500 FAST and HR1™, 384-well LightScanner™ and Roche LightCycler 480. Rotor-Gene 6000, ABI 7500 FAST and Roche LightCycler 480 are capable of performing both qPCR and melt curve analysis. Follow the manufacturer's instruction for data collection and analysis.

4. Expected ΔR and ΔRN: When comparing signal strength among various commercial qPCR Master Mixes, one needs to be mindful of the method used in the comparison. Conventionally, ΔR is the fluorescence gain above the baseline. In general, 10 µL of 1×Eva Green reaction generates higher ΔR than 50 µL 1× Power SYBR from ABI or 1× SYBR Green ER from Invitrogen. ΔRN is defined as ΔR divided by the signal in the ROX channel. Therefore, a higher concentration of ROX will generate smaller ΔRN. ΔRN will also become smaller when ROX is excited at its maximal as in the case of ABI 7500, iCycler IQ, MJ opticon, MJ Chromo4, MX3000, and MX4000. Accordingly, the lower ROX concentration used in some commercial SYBR Green Master Mixes will produce a higher ΔRN.

5. Expected kinetic curve: Based on our comparative studies, amplification curves of Eva Green Master Mix generally are more robust than other commercial Master Mixes formulated using SYBR Green I. Because of SYBR's inhibitory effect, SYBR-based Master Mixes may tend to stall amplification 5-7 cycles after the signal reaches the Ct threshold. In contrast, reactions in EVA Green Master Mix can continue to amplify for as many as 50 cycles.

6. Expected Ct value: Under similar conditions, Ct values generated by Eva Green and SYBR Green I may differ from each other by +1 or –1.

7. Amplicon length: To maximize amplification efficiency with Eva Green Master Mix, the optimal amplicon length is 50-200 bp. For longer amplicons you may need to extend the elongation time.

8. Gel electrophoresis analysis of PCR product: To analyze your PCR product by gel electrophoresis using Eva Green dye in the Master Mix as a prestain, simply add DNA loading buffer your PCR reaction solution, load on a gel, and conduct electrophoresis as usual.
No additional DNA-binding dye needs to be added to either the loading buffer Prior to the experiment, it is prudent to carefully optimize experiment conditions and to include controls at every stage

Recommendations to use Rox Reference Dye 10X

Using Rox Reference dye qPCR Real Time Machine

No ROX	BioRad: iCycler, MyiQ, MiQ 2, iQ 5, CFX-96,CFX-384, MJ Opticon, Option2, Chromo4, MiniOpticon Qiagen: Roto-Gene Q,Roto-Gene3000, Roto-Gene 6000 Eppendorf: Mastercycler realplex Illumina: Eco RealTime PCR SystemCepheid: SmartCyler Roche: LightCycler 480, 96,LightCycler 2.0	**NONE**
Low ROX	ABI: 7500, 7500 Fast, ViiA 7 Stratagene: MX4000P, MX3000P MX3005P, QuantStudio, Illumina Eco	**Dilute 10× ROX 1:10 with dH2O to obtain 1× ROX; add 1 to 2 µL of 1× ROX / 20µL reaction**
High ROX	Applied Biosystems 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast; StepOne, StepOnePlus.	**2 µL of 10× ROX 20 µL reaction**

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