

Eva Green q-PCR Real-Time Master Mix

Superior Performance with an Environmentally Safe Formulation

Product Information

This document contains information for three EvaGreen® qPCR Real Time Master Mix product groups:

1. EvaGreen® qPCR Real Time Master Mix Plus (no ROX)(Cat # FS-T-30032,FS-T-30033)
2. EvaGreen® qPCR Real Time Master Mix Plus with Low ROX (Cat # FS-T-30034 , FS-T-30035)
3. EvaGreen® qPCR Real Time Master Mix with High ROX Plus (Cat# FS-T-30036,FS-T- 30037)

See table below for product information including instrument compatibility related to the product you have purchased.

Table 1. EvaGreen® qPCR RT Master Mix product information and instrument compatibility

Product Group	Cat #	Packaging Size	Component	PCR Instrument
EvaGreen qPCR RT Master Mix Plus (no ROX)	FS-T-30032	400 rxn (4X 1 mL)	EvaGreen® dye, dNTP, buffer composition (including Tris and MgCl ₂) and Cheetah™ hot-start Taq polymerase. Optimized for most of the non-ABI instruments which do NOT require ROX reference dye.	BioRad: iCycler, MyiQ, MiQ 2, iQ 5, CFX-96, CFX-384, MJ Opticon, Option2, Chromo4, MiniOpticon
	FS-T-30033	2000 rxn (20 X 1 mL)		Qiagen: Roto-Gene Q, Roto-Gene3000, Roto-Gene 6000
				Eppendorf: Mastercycler realplex Illumina: Eco RealTime PCR System Cepheid: SmartCycler Roche: LightCycler 480, LightCycler 2.0
EvaGreen qPCR RT Master Mix Plus with Low ROX	FS-T-30034	400 rxn (4X 1 mL)	EvaGreen® dye, dNTP, buffer composition (including Tris and MgCl ₂), Cheetah™ hot-start Taq polymerase and low concentration of ROX reference dye.	ABI: 7500, 7500 Fast Stratagene: MX4000P, MX3000P, MX3005P
	FS-T-30035	2000 rxn (20 X 1 mL)		
EvaGreen qPCR RT Master Mix with High ROX	FS-T-30036	400 rxn (4X 1 mL)	EvaGreen® dye, dNTP, buffer composition (including Tris and MgCl ₂), Cheetah™ hot-start Taq polymerase and high concentration of ROX reference dye.	ABI: 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne, StepOne plus
	FS-T-30037	2000 rxn (20 X 1 mL)		

Storage and Handling

EvaGreen qPCR RT® Master Mix Plus is shipped on blue ice and should be stored immediately upon arrival at -20 °C. When stored in a constant temperature freezer at -20 °C, the kit is stable for at least 12 months from the date of receipt. Before use, thaw at room temperature and mix thoroughly by gentle vortexing. After thawing, the master mix should be kept on ice before use. It can be refrozen for storage.

EvaGreen® Dye Properties

The absorption and fluorescence emission spectra of DNA-bound EvaGreen® dye are very similar to those of SYBR® Green I or FAM: $\lambda_{abs}/\lambda_{em} = 500/530$ nm (DNA bound) (See Figure 1); $\lambda_{abs} = 471$ nm (without DNA).

EvaGreen® dye is the first and *only* qPCR dye that is environmentally safe to dispose down drain due to its inability to cross cell membranes.

Product Description

EvaGreen qPCR RT Master Mix Plus is a ready-to-use hot-start mix for nucleic acid quantitation and melt analysis of PCR amplicons. It delivers clean PCR product even with the most challenging samples tested. Although the master mix is formulated for qPCR using a fast cycling protocol, it is also compatible with qPCR using a regular cycling protocol (see below for recommended cycling protocols of 2-step fast, 3-step fast and universal). ROX reference dye is included in the mix (FS-T-30034 and FS-T-30036 series) for well-to-well fluorescence normalization in ABI and some other instrument platforms. Please refer to the master mix selection guide (Table 1) to decide which master mix is best suited for real time instruments.

A critical component of the master mix is EvaGreen® dye, a unique DNA-binding dye with features ideal for both qPCR and high-resolution melt analysis (HRM).* EvaGreen® dye binds to dsDNA via a novel "release-on-demand" mechanism, which permits saturation dye concentration in qPCR without PCR inhibition. Another important feature of EvaGreen® dye is its safety. DNA-binding dyes are inherently dangerous due to their potential to cause mutation. With this in mind, Biotium's scientists designed EvaGreen® dye such that it cannot cross cell membranes, thus preventing the dye from being in contact with genomic DNA in live cells. All other commercial PCR dyes enter into cells in a matter of minutes. SYBR® Green I, for example, has been shown to be environmentally more toxic than ethidium bromide, a widely known mutagen (Ohta, et al. *Mutation Research*, **492**, 91-97(2001)). Independent labs have confirmed that EvaGreen dye is nonmutagenic, noncytotoxic and safe to aquatic life for direct disposal in the drain. (Visit Biotium website for a full EvaGreen® dye safety report)

Another important component of the master mix is Cheetah™ Taq, our proprietary chemically-modified hot-start DNA polymerase. Unlike AmpliTaq Gold®, which is also a chemically modified Taq but takes 10 minutes or longer to activate, Cheetah™ Taq is fully recovered in 2 minutes with high activity, making it particularly suitable for fast PCR. Cheetah Taq is completely inactive at room temperature and largely free of DNA contamination. This makes Cheetah Taq superior to any antibody-based hotstart Taq, which is typically not completely inactive at room temperature and is prone to DNA contamination due to the nature of antibody production.

The master mix is suitable for two-step mRNA quantitation by first converting mRNA to cDNA via reverse transcription (components not provided), followed by quantitating a portion of the cDNA using the master mix. 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. For accurate quantitation of mRNA level, a none-RT control is recommended to check for the possibility of genomic DNA contamination.

One-step RT-qPCR can also be applied for mRNA quantitation. Primer set 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. Heat-resistant reverse transcriptases that have been tested to be compatible include those from Agilent, Fermentas, Lucigen and Life Technologies. If possible, design primers to have T_m at 55 °C, run both RT step and extension step at 55 °C. For accurate quantitation of mRNA level, a none-RT control is recommended to check for the possibility of genomic DNA contamination.

Another benefit of the EvaGreen qPCR RT® Master Mix Plus is that you can analyze your PCR product by gel electrophoresis without the need to add another DNA-binding dye to either your loading buffer or gel. The EvaGreen® dye in the master mix can act as a DNA prestain, permitting direct and immediate visualization of DNA bands following electrophoresis.

Selected references

1. Khan, et al. Detection of aacA-aphD, qacEδ1, marA, floR, and tetA genes from multidrug-resistant bacteria: comparative analysis of real-time multiplex PCR assays using EvaGreen® and SYBR® Green I dyes, *Molecular and Cellular Probes* (2011), doi: 10.1016/j.mcp.2011.01.004
2. Mao, et al. Characterization of EvaGreen Dye and the implication of its physico-chemical properties for qPCR applications. *BMC Biotechnology* **7**, 76 (2007).
3. Cheng, et al. Detection of hemi/homozygotes through heteroduplex formation in high-resolution melting analysis, *Anal. Biochem.* **410**, 158(2011).
4. White, et al. Methylation-sensitive high-resolution melt-curve analysis of the SNRPN gene as a diagnostic screen for Prader-Willi and Angelman Syndromes. *Clin. Chem.* **53**, 1960 (2007).

Additional Notes:

- 1) qPCR instruments: For iCycler users, you do not need to add FAM to your PCR mix as EvaGreen dye has a slight background fluorescence that provides an adequate and stable baseline level fluorescence; For Roche LightCycler users using glass capillaries for reactions, you need to add BSA (~0.5 mg/mL final concentration). BSA is not necessary if transparent plastic capillary tubes are used.
- 2) Expected ΔR and ΔR_n : 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 μ L of 1X EvaGreen qPCR RT MM reaction generates higher ΔR than 50 μ L 1X PowerSYBR from ABI or 1X SYBR GreenER from Invitrogen. ΔR_n is defined as ΔR divided by the signal in the ROX channel. Therefore, a higher concentration of ROX will generate smaller ΔR_n . ΔR_n will also become smaller when ROX is excited at its peak wavelength as in the case of ABI 7500, iCycler IQ, MJ opticon, MJ Chromo4, MX3000, and MX4000. Accordingly, a lower ROX concentration in a SYBR Green master mix will produce a higher ΔR_n , a technique sometimes used in some of the commercial SYBR Green Kits.
- 3) Expected kinetic curve: Based on our comparative studies, amplification curves of EvaGreen qPCR RT Master Mix are generally more robust than other commercial

* Practicing HRM may require a license from Idaho Technologies, Inc.; SYBR is a trademark of Invitrogen.

PCR Protocols

1. Reaction Setup

Pipet reaction components into each well according to the table below:

Reaction component	Amount required for 20- μ L reaction	Final concentration
2X EvaGreen qPCR RT Master Mix Plus	10 μ L	1X
Primers (See Helpful Tip #1)	x μ L each	0.1-0.5 μ M each
Template (See Helpful Tip #1)	x μ L	
ROX	Optional	See Helpful Tip # 2
H ₂ O	Add to 20 μ L	

Helpful Tips:

- 1) Amplicon length: To maximize amplification efficiency with EvaGreen qPCR RTmaster mix plus, the optimal amplicon length is 50-200 bp. If longer amplicon is necessary, you may need to extend elongation time.
- 2) ROX reference dye: If you are using ABI instruments, ROX is necessary for well-to-well normalization. ROX reference dye is included in the mix (FS-T-30032 and FS-T-30033). See Table 1 on page 1 to decide which master mix is best suited for your instrument.