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November 25, 2014

Product Information

Forget-Me-Not™ qPCR Master Mix

Catalog Number
Without ROX: 31041-T, 31041-1
With Rox 31042-T, 31042-1

Unit Size:
31041-T, 31042-T: 1 mL (100 x 20 uL reactions)
31041-1, 31042-1: 5 mL (500 x 20 uL reactions)

Kit Contents

Component	31041-T	31041-1	31042-T	31042-1
Forget-Me-Not™ qPCR Master Mix (99801)	1 X 1 mL	5 X 1 mL	1 X 1 mL	5 X 1 mL
40X Template Buffer (99802)	1 X 1 mL	2 X 1 mL	1 X 1 mL	2 X 1 mL
ROX Reference Dye (31042C)	N/A	N/A	1 X 1 mL	2 X 1 mL

Storage and Handling
Forget-Me-Not™ qPCR Master Mix is shipped on blue ice and should be stored immediately upon arrival at -20°C. When stored under the recommended condition and handled correctly, the kit should be stable for at least 1 year from the date of receipt. Before use, thaw at room temperature and mix well by gentle vortexing. After thawing, the master mix should be kept on ice before use. It can be refrozen for storage.

Spectral Properties
The absorption and fluorescence emission spectra of DNA-bound EvaGreen® dye are very similar to those of SYBR® Green I or FAM (Figure 1).

λabs/λem = 500/530 nm (DNA bound); λabs = 471 nm (without DNA)

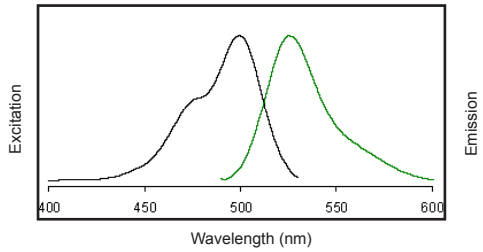

Figure 1. Excitation (left) and emission (right) spectra of EvaGreen® dye bound to dsDNA in pH 7.3 PBS buffer. Also see Ref. 1.

Product Description

Forget-Me-Not™ qPCR Master Mix is a hot-start EvaGreen® based master mix for use in real time PCR applications and DNA melt curve analysis. Its unique combination of a low concentration of a visible blue dye in the reaction mix plus a higher concentration of a visible blue dye in the DNA template buffer minimizes pipetting errors, thereby preventing waste of time, lab reagents, and precious samples (Figure 2). Forget-Me-Not™ qPCR Master Mix has been formulated for fast cycling PCR parameters but can also be used with regular cycling protocols.

Forget-Me-Not™ qPCR Master Mix 31042 kits come with three components. The 2X Master Mix contains EvaGreen® dye, Cheetah™ HotStart Taq DNA Polymerase, dNTPs, and a low concentration of an inert blue dye, which allows the user to distinguish wells containing reaction mix from empty wells. The optional 40X Template Buffer contains a high concentration of inert blue dye, allowing the user to track where DNA templates or water controls have been added to reaction mixes. Finally, the ROX Reference Dye is required for some real time PCR instruments (see Table 1). 31041 kits do not come with ROX, but are otherwise identical to 31042 kits.

Forget-Me-Not™ features EvaGreen® dye, a unique DNA-binding dye with features ideal for both qPCR and High Resolution Melting® (HRM) analysis. EvaGreen® 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 (Ref. 1).

A unique 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 well-known mutagen.[2] Independent labs have confirmed that EvaGreen dye is nonmutagenic, noncytotoxic and safe to aquatic life for direct disposal in the drain. Visit Biotium's website for a full EvaGreen® dye safety report.

An added benefit of an EvaGreen®-based master mix 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 visualization of DNA bands following electrophoresis.

Cheetah™ HotStart Taq DNA Polymerase is Biotium's proprietary chemically-modified hot-start DNA Polymerase. Cheetah™ Taq is fully activated in 2 minutes with high activity recovery, making it particularly suitable for fast PCR. Cheetah™ Taq is completely inactive at room temperature.

Forget-Me-Not™ qPCR Master Mix performs as well as our Fast EvaGreen® Master Mix and as well as or better than Qiagen's QuantiNova® SYBR® Green PCR Master Mix in a real-time PCR assay while also reducing the likelihood of pipetting errors (Figure 3).

PCR Protocols

Reaction component	Amount required per 20 uL reaction	Final concentration
2X Forget-Me-Not™ qPCR Master Mix	10 uL	1X
Primers	x uL each	0.1-0.5 uM each
Template	x uL See Note #1	See Note #2
ROX	Optional	See Note #3 and Table 1
H_2O	Add to 20 uL	

Notes:

1) Template may be added directly, or diluted first in Template Buffer. Template Buffer is provided at 40X and can be diluted to 20X in PCR grade water prior to use. Template Buffer should be at 1X in the final reaction. For example, if 1 uL of DNA template is to be added, a 1:2 dilution of DNA with the 20X Template Buffer should be made and 2 uL added to the final reaction. If 5 uL of DNA template is to be added, 5 uL of DNA would be added to 1 uL 20X Template Buffer, then 6 uL total would be added to the final reaction. The use of Template Buffer is optional, but all reactions in a given experiment should contain the same amount for accurate comparisons. **Important Note:** Template Buffer should be well thawed and vortexed prior to use, and care should be used during pipetting to ensure no dye sticks to the outside of the pipette tip or is left remaining inside the tip.

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 for the recommended ROX concentration for your instrument (minor adjustments may be needed). ROX may add noise to melt curve analysis, which could be mistaken for real peaks. Thus, in case of unexpected 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.

Cycling Protocols

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

A. Two-step fast cycling protocol

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

Cycling Step	Temperature	Holding Time	Number of Cycles
Enzyme activation	95 °C	2 min	1
Denaturation	95 °C	5 s	45
Annealing & Extension	60 °C	30 s	

B. 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	Temperature	Holding Time	Number of Cycles
Enzyme activation	95 °C	2 min	1
Denaturation	95 °C	5 s	45
Annealing	50-60 °C	5 s	
Extension	72 °C	25 s	

C. 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 under fast cycling conditions.

Cycling Step	Temperature	Holding Time	Number of Cycles
Enzyme activation	95 °C	2 min	1
Denaturation	95 °C	15 s	45
Annealing & Extension	60 °C	60 s	

General Considerations

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 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.

3) Amplicon length: To maximize amplification efficiency with Fast EvaGreen® master mix, the optimal amplicon length is 50-200 bp. For longer amplicons you may need to extend the elongation time.

4) Gel electrophoresis analysis of PCR product: To analyze your PCR product by gel electrophoresis using the EvaGreen® dye in the master mix as a prestain, simply add DNA loading buffer to 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 or the gel. Gel visualization can be carried out using a 254 nm UV box, or a gel imager or Dark Reader using a SYBR® Green filter. Alternatively, the gel may be imaged using a 488 nm laser-based gel scanner.

References

1. Mao, et al. Characterization of EvaGreen Dye and the implication of its physico-chemical properties for qPCR applications. BMC Biotechnology 7, 76-91 (2007).

2. Ohta, et al. Ethidium bromide and SYBR Green I enhance the genotoxicity of UV-irradiation and chemical mutagens in E. coli. Mutation Res. 492, 91-97 (2001).

Table 1. Recommended ROX Concentration for PCR Instruments

PCR Instrument	Recommended Rox Concentration	Amount of ROX per 20 uL reaction
BioRad: iCycler, MyiQ, MiQ 2, iQ 5, CFX-96, CFX-384, MJ Opticon, Option2, Chromo4, MiniOpticon Qiagen: Rotor-Gene Q, Rotor-Gene3000, Rotor-Gene 6000 Eppendorf: Mastercycler realplex Illumina: Eco RealTime PCR System Cepheid: SmartCyler Roche: LightCycler 480, LightCycler 2.0	No ROX	None
ABI: 7500, 7500 Fast, ViiA 7 Stratagene: MX4000P, MX3000P, MX3005P	Low ROX	If using Template Buffer, dilute ROX 1:10 with dH_2O and add 1.8 uL diluted ROX per 20 uL reaction.
		If not using Template Buffer, dilute ROX 1:100 with dH_2O and add 3 uL diluted ROX per 20 uL reaction.
ABI: 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne, StepOne plus	High ROX	If using Template Buffer add 2uL ROX Reference Dye per 20 uL reaction.
		If not using Template Buffer, dilute ROX 1:10 with dH_2O and add 3 uL ROX Reference Dye per 20 uL reaction.

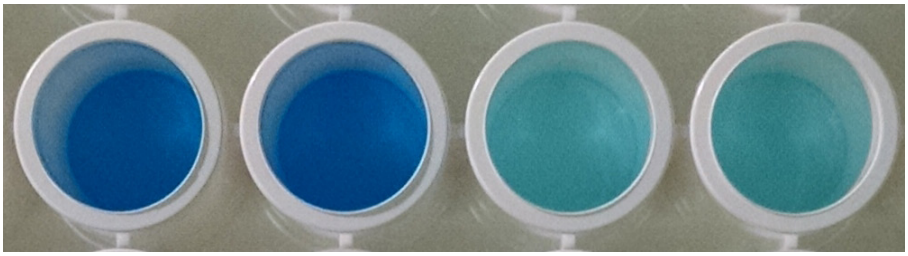

Figure 2: Left two wells contain qPCR reaction mix including Forget-Me-Not™ Master Mix and have had the DNA template or NTC control added in Template Buffer, hence the darker blue color. The right two wells have not yet had the DNA template or NTC control in Template Buffer added, and therefore are still the lighter blue of the 1X Forget-Me-Not™ Master Mix.

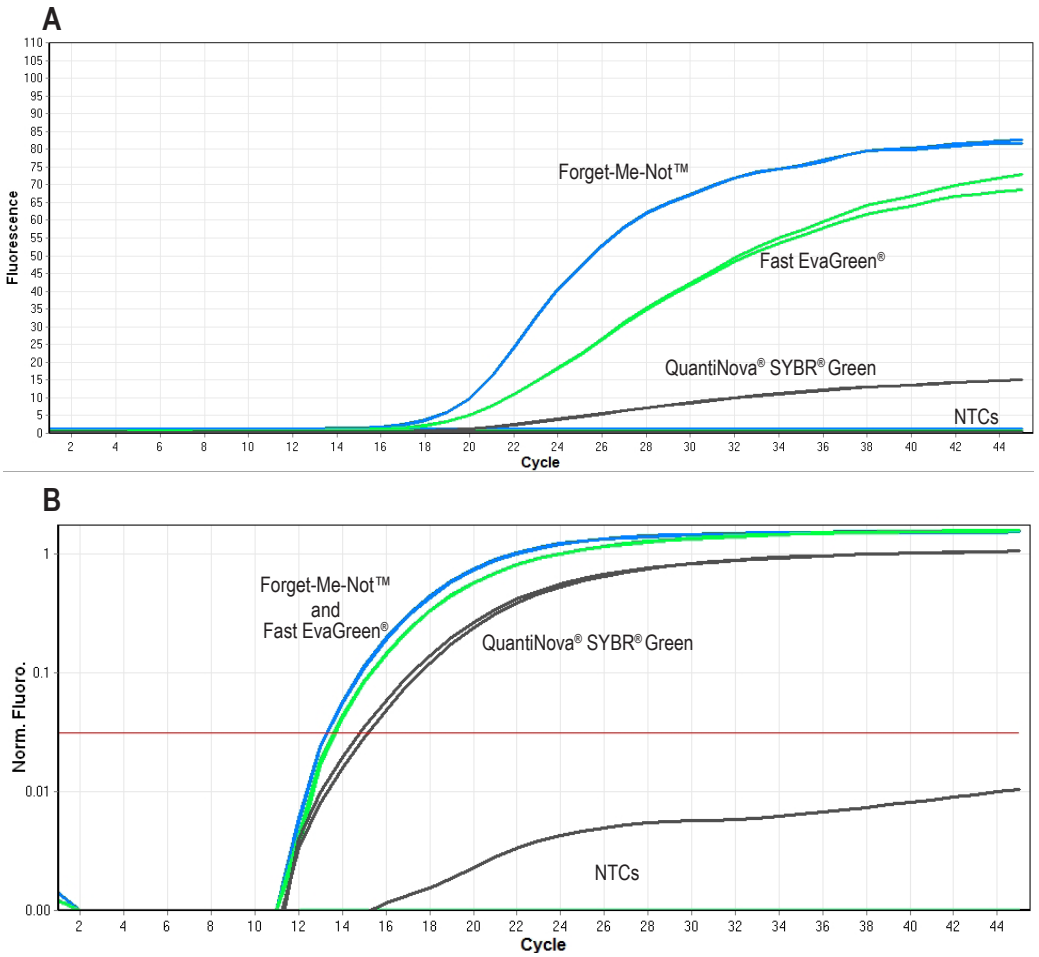

Figure 3: Raw data in linear (A) and logarithmic scale (B) from a real-time PCR assay comparing Forget-Me-Not™, Fast EvaGreen®, and Qiagen's QuantiNova® SYBR® Green PCR Kit. Note higher relative fluorescent signal from EvaGreen®-based master mixes.

Related Products

Catalog number	Product	Size
31003	Fast EvaGreen qPCR Master Mix (200 rxn)	2 x 1 mL
31000-T	EvaGreen Dye, 20X in water	1 mL
31020	Fast Plus EvaGreen qPCR Master Mix (200 rxn)	2 x 1 mL
31014	Fast Plus EvaGreen qPCR Master Mix, low ROX (200 rxn)	2 x 1 mL
31015	Fast Plus EvaGreen qPCR Master Mix, high ROX (200 rxn)	2 x 1 mL
31005	Fast Probe Master Mix (no ROX) (200 rxn)	2 x 1 mL
31016	Fast Probe Master Mix (with ROX) (200 rxn)	2 X 1 mL
29050	Cheetah HotStart Taq DNA Polymerase	500 U
29054	HotStart Polymerase Modification Kit	1 kit
40013	PMA Dye	1 mg
40019	PMA Dye 20 mM in water	100 uL

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