September 13, 2017

Product Information

Forget-Me-Not™ EvaGreen® qPCR Master Mix

Catalog Number
Without ROX: 31041-T, 31041-1, 31041-20mL
With Rox 31042-T, 31042-1, 31042-20mL

Unit Size
31041-T, 31042-T: 1 mL (100 x 20 uL reactions)
31041-1, 31042-1: 5 mL (500 x 20 uL reactions)
31041-20mL, 31042-20mL: 20 mL (2000 x 20 uL reactions)

Kit Contents

Kits without ROX:

Component	31041-T	31041-1	31041-20mL
2X Forget-Me-Not™ EvaGreen® qPCR Master Mix (99801)	1 X 1 mL	5 X 1 mL	2 X 10 mL
40X Template Buffer (99802)	1 X 1 mL	2 X 1 mL	1 X 8 mL

Kits with ROX:

Component	31042-T	31042-1	31042-20mL
2X Forget-Me-Not™ EvaGreen® qPCR Master Mix (99801)	1 X 1 mL	5 X 1 mL	2 X 10 mL
40X Template Buffer (99802)	1 X 1 mL	2 X 1 mL	1 X 8 mL
ROX Reference Dye (31042C)	1 X 0.2 mL	1 X 1 mL	1 X 4 mL

Storage and Handling
Forget-Me-Not™ EvaGreen® qPCR Master Mix is shipped on blue ice and should be stored at -20°C upon arrival. Store protected from light. When stored as recommended the product is 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, or kept at 4°C for 1 week without loss of activity.

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

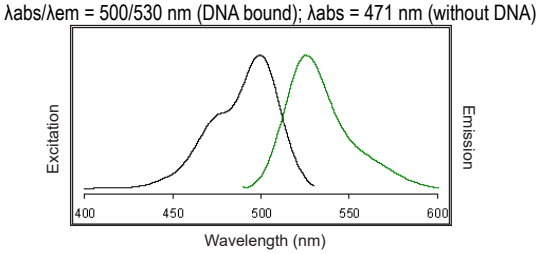

Figure 1. Excitation (left) and emission (right) spectra of EvaGreen® dye bound to dsDNA in PBS. Also see Ref. 1.

EvaGreen® dye is safer than SYBR® Green I. DNA-binding dyes are inherently dangerous due to their potential to cause mutation, but EvaGreen® dye cannot cross cell membranes, thus preventing it from coming in contact with genomic DNA in live cells. Independent labs have confirmed that EvaGreen dye is nonmutagenic, noncytotoxic and safe to aquatic life for direct disposal down the drain. Visit Biotium's website for a full EvaGreen® dye safety report.

Product Description
Forget-Me-Not™ qPCR Master Mix is a 2X hot-start EvaGreen® based master mix for use in real-time PCR applications and DNA melt curve analysis. The master mix has been formulated for fast cycling PCR parameters but can be used with regular cycling protocols.

The 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 visually 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. This unique combination of a low concentration of a visible blue dye in the reaction mix plus a higher concentration of a visible blue dye (Figure 2) in the DNA template buffer minimizes pipetting errors, thereby preventing waste of time, lab reagents, and precious samples.

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

Reaction Setup

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 DNA	x uL[a]	See note [b]
ROX / Well calibrator	Optional	See note [c] and Table 1
H_2O	Add to 20 uL	

[a] 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.

[b] 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. 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 µg RNA), but the RT reaction volume must not exceed 10% of the final PCR volume.

[c] 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.

Note: Template Buffer quenches ROX fluorescence. Refer to Table 1 for the recommended ROX concentrations when Template Buffer is used.

Bio-Rad's iCycler 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.

Cycling Protocols

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 primer Tm's are designed to be 60 °C.

Cycling Step	Temperature	Holding Time	Number of cycles
Enzyme activation	95 °C	2 min	1
Denaturation	95 °C	2-5 sec	40
Annealing / extension / data acquisition	60 °C	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	Temperature	Holding Time	Number of cycles
Enzyme activation	95 °C	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		

General Considerations

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

2) EvaGreen® dye can be used for high resolution melting (HRM) analysis. Follow the qPCR system's instructions for data collection and analysis.

3) Gel electrophoresis analysis of PCR product: After PCR with EvaGreen® 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.

4) Roche LightCycler users, using glass capillaries for reactions, should add BSA to the PCR reactions at a ~0.5 mg/mL final concentration. BSA is not necessary if plastic capillary tubes are used.

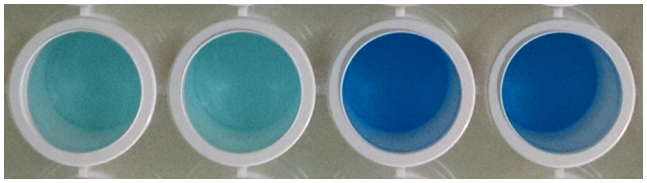

Figure 2: The two wells on the left contain 1X Forget-Me-Not™ Master Mix. The light blue color is useful for distinguishing empty wells from wells containing reaction mix. The wells on the right contain the reaction mix to which DNA mixed with Template Buffer has been added.

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 Touch™, CFX-384 Touch™ and Connect™, Chromo4™, MiniOpticon™ Qiagen: Rotor-Gene® Q, Rotor-Gene® 3000, 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™, QuantStudio™ 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. 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 dilute ROX 1/10 with dH2O and add 30 uL per 1 mL tube of master mix.
ABI: 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne®, StepOnePlus®	High ROX	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.

Related Products

Catalog number	Product
31043-1	Forget-Me-Not™ Universal Probe Master Mix
31000-T	EvaGreen Dye, 20X in water
31003	Fast EvaGreen qPCR Master Mix (200 rxn)
29050	Cheetah HotStart Taq DNA Polymerase
29054	HotStart Polymerase Modification Kit
40069	PMAxx, 20 mM in water, for viability PCR
E90003	Gel-Bright™ LED Gel Illuminator
E90002	PMA-Lite™ LED Photolysis Device
41003	GelRed™ Nucleic Acid Gel Stain, 10,000X in water
31039	1 kb DNA Ladder in TE Buffer
31040	100 bp DNA Ladder in TE Buffer

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).

Please visit our website at www.biotium.com for information on our life science research products, including environmentally friendly EvaGreen® qPCR master mixes, fluorescent CF™ dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.