August 23, 2024

Product Information

Forget-Me-Not™ EvaGreen® qPCR Master Mix (2-Color Tracking)

Kits without ROX:

Component	31041-T 100 x 20 uL reactions	31041-1 500 x 20 uL reactions	31041-20mL 2000 x 20 uL reactions
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 supplied with a separate tube of ROX:

Component	31042-T 100 x 20 uL reactions	31042-1 500 x 20 uL reactions	31042-20mL 2000 x 20 uL reactions
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

Standalone Master Mix (no ROX, no Template Buffer)

Component	99801-10mL 1000 x 20 uL reactions
2X Forget-Me-Not™ EvaGreen® qPCR Master Mix (99801)	1 x 10 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.

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 tracking dyes helps minimize pipetting errors to avoid wasted time, reagents, and precious samples. We also supply 2X Master Mix (99801-10ML) for standalone purchase for customers who do not wish to use template buffer.

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 (Figure 1) are very similar to those of SYBR® Green I or FAM with Ex/Em at 500/530 nm with DNA.

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 non-mutagenic, non-cytotoxic and safe to aquatic life for direct disposal down the drain. Visit Biotium’s website for a full EvaGreen® Dye safety report.

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.

References

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

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.
- EvaGreen® Dye can be used for high resolution melting (HRM) analysis. Follow the qPCR system's instructions for data collection and analysis.
- Gel electrophoresis analysis of PCR products: 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.
- 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. We also offer Forget-Me-Not™ EvaGreen® qPCR Master Mix pre-mixed with low ROX or high ROX (see Related Products).
- Instruments that do not require ROX reference dye are generally compatible with qPCR master mixes containing ROX (check with the manufacturer before use).
- 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 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 ug RNA), but the RT reaction volume must not exceed 10% of the final PCR volume.
- 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.

Table 1. Instrument Compatibility

PCR Instrument	Recommended ROX Concentration	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 Forget-Me-Not™ EvaGreen® qPCR Master Mix without ROX (Cat. No. 31041).

Protocols for Use

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 Tm of the primers is designed to be 60°C.

Cycling Step	Temp	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	Temp	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		

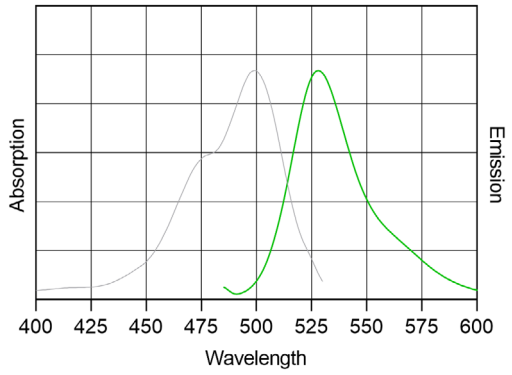

Figure 1. Absorption (left) and emission (right) spectra of EvaGreen® Dye bound to dsDNA in PBS. Also see Ref. 1.

Related Products

Cat. No.	Product
31043	Forget-Me-Not™ Universal Probe qPCR Master Mix
31045	Forget-Me-Not™ EvaGreen® qPCR Master Mix (Low ROX)
31046	Forget-Me-Not™ EvaGreen® qPCR Master Mix (High ROX)
31078	N-Flux™ 5X Digital PCR Master Mix
31000	EvaGreen® Dye, 20X in Water
31077	EvaGreen® Plus Dye, 20X in Water
29050	Cheetah™ HotStart Taq DNA Polymerase
31079	EvaRuby™ Dye, 20X in Water
29087-50uL	VeriFluor™ Far-Red Passive Reference Dye, 400X in Water
29054	HotStart Polymerase Modification Kit
29051	EvaEZ™ Fluorometric Polymerase Activity Assay Kit
41003	GelRed® Nucleic Acid Gel Stain, 10,000X in Water
41005	GelGreen® Nucleic Acid Gel Stain, 10,000X in Water
31085	100 bp DNA Ladder, Ready-to-Load
31084	1 kb DNA Ladder, Ready-to-Load
41024-4L	Water, Ultrapure Molecular Biology Grade
41006	TBE Buffer, 5X
E90005	Gel-Bright™ Laser Diode Gel Illuminator

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.