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## Forget-Me-Not™ EvaGreen® qPCR Master Mix (Low ROX or High ROX)



A high-performance dye-based qPCR master mix containing EvaGreen® qPCR dye, Cheetah™ HotStart Taq Polymerase, and Forget-Me-Not™ tracking dye. With low or high ROX.

### Product attributes

Detection method/readout	PCR/qPCR, HRM®
Reference dye	Low ROX, High ROX

### Product Description

2X Forget-Me-Not™ EvaGreen® qPCR Master Mix is a ready-to-use high performance dye-based master mix for qPCR and DNA melt curve analysis. The 2X Master Mix contains EvaGreen® dye, premixed ROX, Cheetah™ HotStart Taq DNA Polymerase, dNTPs, and a low concentration of the Forget-Me-Not™ blue tracking dye.

- Ready-to-use, just add primers and DNA template
- Optimized with our superior EvaGreen® Dye which is more sensitive than SYBR® Green I and other commercial PCR dyes
- Prevent wasted samples and other costly mistakes with our Forget-Me-Not™ light blue master mix
- Premixed with ROX and optimized for instruments requiring either high or low concentrations of ROX reference dye
- Formulated with Cheetah™ HotStart Taq for fast cycling protocols
- Directly visualize amplification products in a gel

### Forget-Me-Not™ Color Tracking

Catch pipetting mistakes and avoid wasting time, reagents, and your precious DNA samples. Forget-Me-Not™ EvaGreen® qPCR Master Mixes with premixed ROX, and has a light blue color so you can easily distinguish tubes/wells containing reaction mix from empty tubes/wells (Figure 1). This product is for users who wish to use single color tracking. We also offer [Forget-Me-Not™ EvaGreen® qPCR Master Mix \(2-Color Tracking\)](#) which allows color tracking of both master mix and template solution.

### Peace of Mind Plus PCR Performance

Forget-Me-Not™ qPCR Master Mix is formulated for fast cycling PCR parameters, but can also be used with regular cycling protocols. Forget-Me-Not™ qPCR Master Mix performs as well as our Fast EvaGreen® Master Mix, and as well or better than Qiagen's QuantiNova® SYBR® Green PCR Master Mix in a real-time PCR assay (Figure 2), while making it easy for you to make sure your reactions are set up correctly the first time, every time.

### EvaGreen® Dye

An environmentally friendly, non-mutagenic, and non-cytotoxic DNA-binding dye with features ideal for both qPCR and High Resolution Melt® (HRM) analysis (Ref. 3). EvaGreen® Dye binds to dsDNA via a novel "release-on-demand" mechanism in which the dye is in an inactive conformation until DNA is available. As DNA is produced during the PCR process, the dye shifts to an active conformation that binds DNA to emit fluorescence. As a result, EvaGreen® Dye can be used at a much higher dye concentration in qPCR than SYBR® Green I (Ref. 1). The dye has excitation and emission spectra very close to those of fluorescein (FAM) or SYBR® Green I, making it readily compatible with instruments equipped with the 488 nm argon laser or any visible light excitation with wavelength in the region. EvaGreen® amplification products can be directly detected on a gel without the need for another DNA gel stain.

Independent labs have confirmed that EvaGreen® dye is non-mutagenic, non-cytotoxic, and safe to aquatic life for direct disposal in the drain. 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 (Ref. 2). For details, download the [EvaGreen® Dye Safety Report](#).

Learn more about [EvaGreen® Dye for qPCR](#).

### Cheetah™ HotStart Taq

Cheetah™ HotStart Taq DNA Polymerase is Biotium's proprietary chemically-modified hotstart DNA Polymerase. Cheetah™ Taq is completely inactive at room temperature, and is fully activated after 2 minutes at 95°C, making it particularly suitable for fast cycling PCR protocols.

### ROX

Forget-Me-Not™ EvaGreen® qPCR Master Mix with Premixed ROX is available in two formulations: a low ROX master mix (~50 nM final ROX concentration in assay) and a high ROX master mix (~500 nM final assay concentration). ROX is required as a passive reference dye in some qPCR systems to compensate for well-to-well optical variations. Refer to the table below for the passive dye requirements of real-time PCR instruments.

Reference Dye	PCR Instrument
Low ROX (~50 nM)	Applied Biosystems®: 7500, 7500 Fast, ViiA™7, Quant-Studio™ instruments Stratagene (Agilent): MX4000P, MX3000P, MX3005P
High ROX (~500 nM)	Applied Biosystems®: 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™, StepOnePlus™
No ROX required*	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, Rotor-Gene® 6000 Eppendorf: Mastercycler® Realplex Illumina: Eco™ RealTime PCR System Cepheid: SmartCycler® Roche: LightCycler® 480, LightCycler® 2.0
Fluorescein**	BioRad: iCycler™, MyiQ™, MiQ™, iQ™

\*Instruments that do not require ROX reference dye are generally compatible with qPCR master mixes containing ROX (check with the manufacturer before use). We also sell [Forget-Me-Not™ EvaGreen® qPCR Master Mix kits without ROX \(cat# 31041\)](#), and with a separate tube of ROX ([cat# 31042](#)) that can be used to make your own low or high ROX master mixes.

\*\*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. For these instruments we recommend using [Forget-Me-Not™ EvaGreen® qPCR Master Mix without ROX \(cat# 31041\)](#).

EvaGreen Dye and Cheetah Taq are covered under US and international patents. QuantiNova is a registered trademark of Qiagen Group. SYBR is a registered trademark of Thermo Fisher Scientific. HRM is a registered trademark of Idaho Technologies, Inc./BioFire Defense, LLC; the use of HRM may require a license.

## References

1. BMC Biotechnol (2007) <https://doi.org/10.1186/1472-6750-7-76>
2. Mutation Res. (2001) [https://doi.org/10.1016/S1383-5718\(01\)00155-3](https://doi.org/10.1016/S1383-5718(01)00155-3)
3. Parasites Vectors (2019) <https://doi.org/10.1186/s13071-019-3781-4>

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