

Real Time PCR (RT-PCR, qPCR)

EvaGreen

EvaGreen®, the Next-Generation PCR Dye

EvaGreen® dye is a next-generation DNA-binding dye with features ideal for use in quantitative real-time PCR (qPCR) and many other applications. Biotium scientists designed the dye by taking into consideration several essential dye properties relevant to PCR, including PCR inhibition, safety, stability and fluorescence spectra of the dye. The result of our efforts is a dye superior to SYBR® Green I and other commercial PCR or high-resolution melt curve (HRM) dyes.*

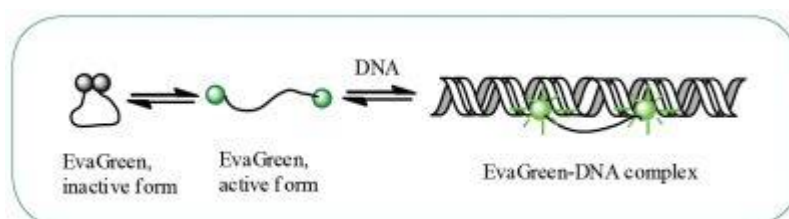


Figure 1. EvaGreen® dye binds to dsDNA via a novel “release-on-demand” mechanism.



Figure 2. EvaGreen® is non-toxic, non mutagenic and non hazardous to aquatic life.

EvaGreen® dye combines superior brightness and sensitivity, a great solution for dye-based qPCR master mixes with the ability to do sensitive melt curve analysis in the same reaction. In addition, EvaGreen® dye is non-toxic, non-mutagenic, and not hazardous to aquatic life.

Mechanism of DNA Binding

EvaGreen® DNA-binding dye is designed using a novel concept of DNA binding via “release-on-demand” mechanism (Fig. 1). The dye is constructed of two monomeric DNA-binding dyes linked by a flexible spacer. In the absence of DNA, the dimeric dye assumes a looped conformation that is inactive in DNA binding. When DNA is available, the looped conformation shifts via an equilibrium to a random conformation that is capable of binding to DNA to emit fluorescence. This chemical equilibrium provides a

unique mechanism to continuously supply the active form of the dye from the “reserve”(i.e., the dye in looped conformation), as more DNA is formed during a PCR process. Consequently, an EvaGreen® master mix can be formulated with relative high dye concentration to maximize fluorescence signal without PCR inhibition, making the mix suitable for both qPCR and HRM applications.*

Features

- **Environmentally safe**
Non-mutagenic, non-cytotoxic and safe to aquatic life for safe handling and easy disposal down the drain.
- **Superior for qPCR and isothermal amplification**
Far brighter than SYBR® Green I for detecting amplification due to its novel “release-on-demand” DNA-binding mechanism.
- **Unrivaled DNA melt curve performance**
Low PCR inhibition permits the use of saturating dye concentration for maximal signal and high-resolution DNA melt analysis.
- **Extremely stable**
Stable during storage or under PCR conditions.
- **Serves both as a qPCR dye and a DNA gel stain**
After the PCR reaction, directly visualize the PCR product on a gel using a UV transilluminator or blue light box without the need for another gel stain.
- **Compatible with multiplex PCR**
Lack of dye migration from amplicon to amplicon enables detection of multiple PCR products by melt curves.
- **Useful for other applications**
Currently the only qPCR dye to be used in droplet digital PCR (ddPCR). EvaGreen® can also be used for isothermal amplification, microfluidic PCR systems, capillary gel electrophoresis, and more.

Ordering information:

www.interchim.com⁰

EVAGREEN Dye, 2000X in DMSO
31019 , 50µL

EVAGREEN Dye, 20X in water
31000(5x1ml), 31000-T(1ml trial size)

see more: [EvaGreen qPCR Master Mixes](#)

Superior PCR Performance

A PCR dye emits fluorescence by forming a dye-DNA complex. The interaction with DNA inevitably leads to some PCR interference. PCR inhibition by the dye can be particularly serious at the early stage of PCR, where the dye-to-DNA ratio is high. On the other hand, a sufficient dye concentration is important for generating good signal. Thus, an optimal dye concentration must be used in order to attain reliable PCR performance.

For many current DNA-binding PCR dyes, such as SYBR® Green I, the optimal dye concentration can be quite low, which limits PCR signal and also makes the dyes unsuitable for high-resolution melt curve (HRM) analysis. Furthermore, a master mix with low SYBR® Green concentration may fail to detect multiple amplicons by melt peaks due to dye migration from small amplicons to large amplicons, giving the false result of a clean single amplicon for a PCR that may, in fact, produce several products.

To these points, Evagreen dye is designed to yield superior results:

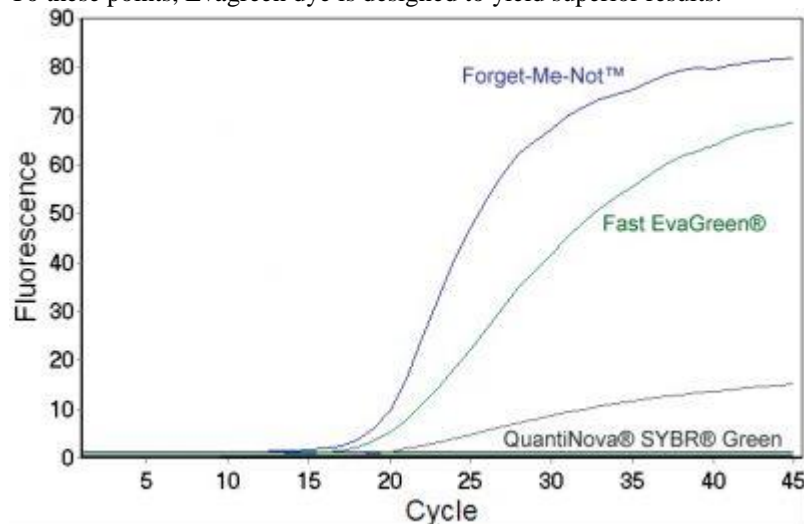


Figure 3. A comparison of the raw fluorescence signal from qPCR reactions performed with two EvaGreen® master mixes (Forget-Me-Not™ EvaGreen® and Fast EvaGreen®) and QuantiNova SYBR® Green. EvaGreen® dye is less inhibitory than SYBR® green, allowing for a much brighter signal.

EvaGreen® Dye Safety

Another major advantage of EvaGreen® dye over other PCR and HRM dyes is its safety. EvaGreen® dye is the first and only PCR dye to date designed to be environmentally safe.

Very few PCR dyes have been thoroughly studied for their safety despite the increasing use of PCR in research and diagnostics and the fact that DNA-binding dyes are inherently dangerous due to their potential to cause mutation.

Thus, handling and disposal of PCR master mixes can be a health and environmental issue. Indeed, SYBR® Green I is found to be even more environmentally toxic than ethidium bromide, one of the best known mutagens. SYBR® Green I has been suggested to interfere with DNA repair mechanisms in cells, and as a result it potentiates genotoxicity of chemicals as well as DNA damage by UV light.

Although no safety data are available on other PCR and HRM dyes (e.g., SYTO9, LC Green, BRYT Green and ResoLight), those dyes are all known to enter cells in a matter of minutes, thus posing potential genotoxicity risk. With this in mind, EvaGreen® dye is designed to be cell membrane impermeable by increasing the molecular size and charge of the dye (Figure 4). Because EvaGreen® dye is denied the chance to interact with genomic DNA in living cells, it is made much safer than the other dyes. Independent laboratory tests have confirmed that EvaGreen® is nonmutagenic, noncytotoxic and safe to aquatic life. The dye has passed environmental hazardous waste regulation in the state of California (CCR title 22) for easy disposal down the drain. [Ask](#) the EvaGreen Safety Report for more information.

EvaGreen® Dye Stability

EvaGreen® dye is very stable both during storage and under PCR conditions. EvaGreen dye has been reported to be stable at temperatures as high as 65°C for up to 6 months of use in the field for real-time PCR (Mil Med. 2014 179(6):626-32).

SYBR® Green I, on the other hand, is known to degrade following multiple freeze-thaw cycles and under PCR conditions. Moreover, decomposed SYBR® Green I is reported to be even more inhibitory to PCR. Thus, when assessing the performance of an EvaGreen-based master mix, you can eliminate the stability of the dye as a variable.

Spectral Compatibility

EvaGreen® dye is spectrally similar to FAM or SYBR® Green I, which means no change in optical settings is required for using an EvaGreen-based master mix.

Other EvaGreen® dye applications

EvaGreen® dye is currently the only qPCR dye to be used in droplet digital PCR (ddPCR).

EvaGreen® dye has been applied in numerous other applications, such as Fluidigm® microfluidic PCR systems, isothermal amplification, capillary gel electrophoresis, DNA quantitation in solution and selective detection of dead cells in cell viability tests. Biotium offers EvaGreen® stand-alone dye, EvaGreen® qPCR master mixes, and other reagents and kits for qPCR.

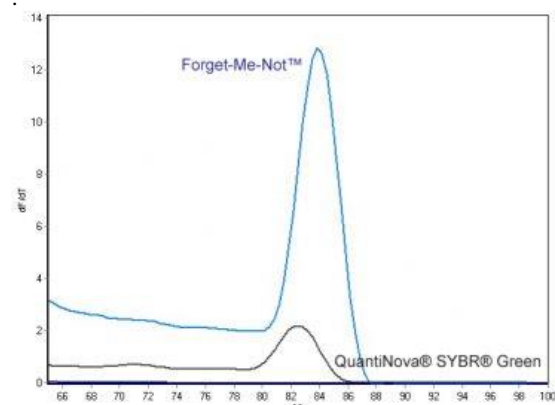


Figure 5. Melt curve analysis of a GAPDH qPCR product using either an EvaGreen® master mix (Forget-Me-Not™ EvaGreen®) or a SYBR® Green master mix (QuantiNova SYBR® Green). EvaGreen® Dye is less inhibitory to PCR than SYBR® Green, allowing you to use more dye, and thus achieve a stronger melt curve signal.

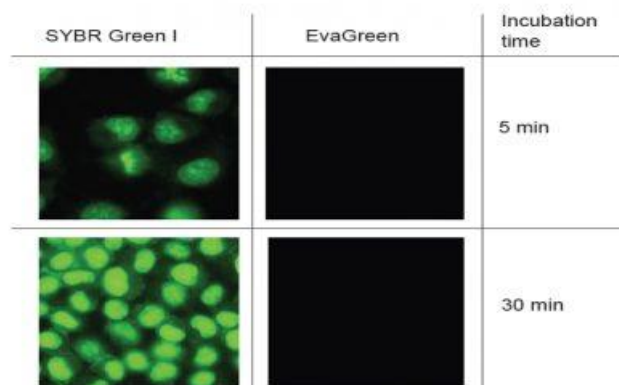


Figure 4. EvaGreen® Dye is safer than SYBR® Green I due to the fact that it is cell membrane impermeable, and therefore cannot gain access to the DNA in the nuclei of living cells.

Forget-Me-Not qPCR Master Mixes

- **Economic**
- EvaGreen® Dye – **better signal than SYBR® Green, safer and more environmentally friendly**
- Cheetah™ Hotstart Taq – **fully activated after two minutes at 95°C for fast cycling protocols**
- Available with a **2-color tracking system** to help to catch pipetting mistakes, saving time, reagents, DNA samples, and money
- Available without **ROX passive dye**, with a separate tube of ROX, or premixed with ROX (high or low concentrations)



Figure 1. The Forget-Me-Not™ EvaGreen® Master Mix (2-color tracking) containing a light blue dye before (left) and after (right) addition of template containing the dark blue template buffer.

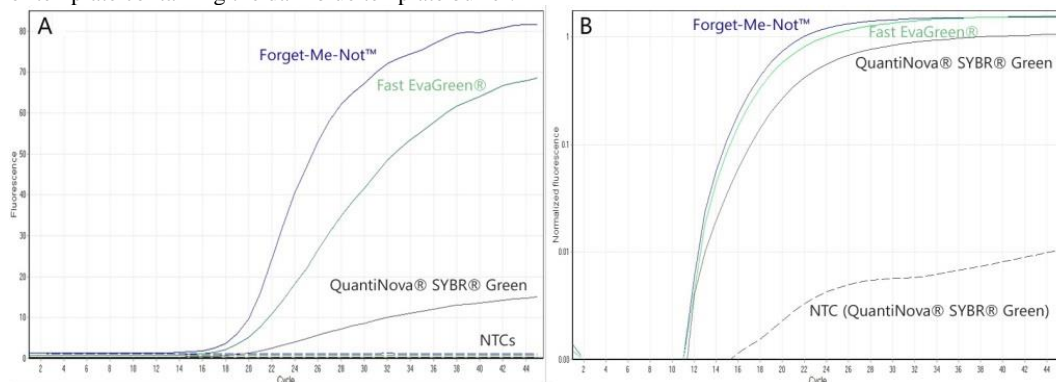


Figure 2. Forget-Me-Not™ EvaGreen® qPCR master mix performs as well or better than other dye-based PCR master mixes, with exceptional brightness and sensitivity.

EvaGreen® dye

A unique DNA-binding dye with features ideal for both qPCR and High Resolution Melting® (HRM) analysis*. See [more information above](#).

Cheetah™ HotStart Taq

Cheetah™ HotStart Taq DNA Polymerase is Biotium's proprietary chemically-modified hot-start 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.

Ordering information: www.interchim.com⁰

Forget-Me-Not EvaGreen qPCR Master Universal Mix

Catalog #: 31043-T(100reactions), 31043-1(500reactions)

Forget-Me-Not EvaGreen qPCR Master Universal Mix with ROX

Catalog #: 31044-T(100reactions), 31044-1(500reactions)

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

Catalog #: 31041-T, 31041-1, 31041-20mL, 31042-T, 31042-1, 31042-20mL

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

Catalog #: 31045-1mL, 31045-5mL, 31045-20mL, 31046-1mL, 31046-5mL, 31046-20mL

Ordering information: www.interchim.com⁰

• EvaGreen Dye

EVAGREEN Dye, 2000X in DMSO 31019 , 50µL
EVAGREEN Dye, 20X in water 31000(5x1ml), 31000-T(1ml trial size)

• EvaGreen qPCR Master mixes

FAST EVAGREEN MASTER MIX FOR QPCR & HRM	31003, 2 x 1 ml	31003-T, 1 ml trial size
	31003-1, 5 x 1 ml	31003-2, 50 x 1 ml
FAST Plus EVAGREEN MASTER MIX	31020, 2 x 1 ml	31020-T, 1 ml trial size
	31020-1, 5 x 1 ml	31020-2, 50 x 1 ml
FAST Plus EVAGREEN MASTER MIX Low ROX	31014, 2 x 1 ml	31014-T, 1 ml trial size
	31014-1, 5 x 1 ml	31020-2, 50 x 1 ml

• See also **Forget-Me-Not qPCR Master Mixes (uses EvaGreen dye)**

Related products and documents:

PH-BD066b, Clonage – restriction enzymes

Information inquire

Reply by Fax : +33 (0) 4 70 03 82 60 or email at interbiotech@interchim.com

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