

EvaGreen™

The very best dye for qPCR and HRM

FEATURES

■ Highly sensitive

Produces the most robust PCR signal when used at the recommended concentration.

■ Low PCR inhibition

Exhibits much less PCR inhibition than SYBR® Green I via a smart "release-on-demand" DNA-binding technology.

■ Compatible with Fast PCR protocol

Minimal interference to PCR makes it possible to significantly shorten the chain extension time.

■ Excellent for HRM application

Lack of "dye redistribution" problem makes it compatible with post-PCR high-resolution melt curve analysis (HRM) in a closed-tube format.

■ Compatible with multiplex PCR

No dye migration from amplicon to amplicon when used at the recommended concentration.

■ Extremely stable

Simply indestructible under most biochemical conditions. Can be stored at room temperature and be subject to repeated freeze-thaw cycles.

■ Spectrally similar to SYBR® Green I

Compatible with all major brand qPCR instruments.

■ Nonmutagenic and noncytotoxic

* EvaGreen™ and its uses are covered by pending US and international patents; SYBR is a registered trademark of Molecular Probes, Inc.



EvaGreen vs. Its Competitions

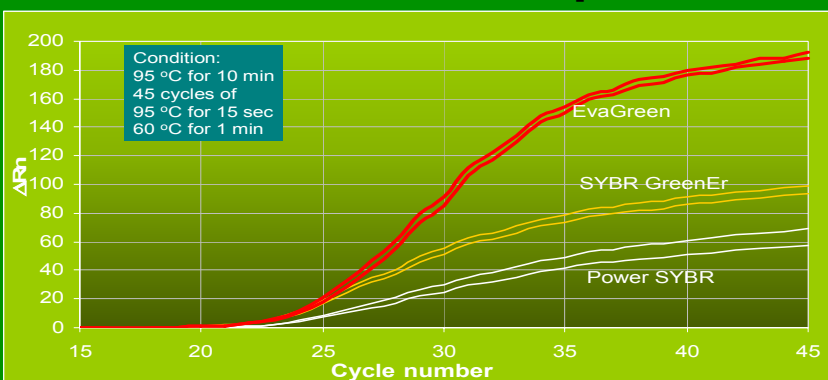


Figure 1. Amplifications of a Myc gene fragment (75 ng huDNA input) (ABI) using EvaGreen™ (red), SYBR® GreenER™ (green) (Invitrogen) and Power SYBR® (yellow) (ABI), respectively. All reactions were run in duplicate on an ABI 7900 instrument using the Universal cycling protocol (15 s at 95 °C and 60 s at 60 °C).

EvaGreen™ is a DNA-binding dye with many features that make it a superior alternative to SYBR® Green I for quantitative real-time PCR (qPCR). Apart from having similar spectra, EvaGreen™ has three important features that set it apart from SYBR® Green I.

First, EvaGreen™ has much less PCR inhibition than SYBR® Green I. As a result, qPCR employing EvaGreen™ may be run using a fast PCR protocol (Figure 2). Moreover, EvaGreen™ can be used at a relatively high concentration, which results in a significantly higher PCR signal than that with SYBR® Green I (Figure 1). The relatively high EvaGreen™ concentration also eliminates so-called "dye redistribution" problem, making EvaGreen™ suitable for both PCR multiplexing and high-resolution melt curve analysis (HRM) (Figure 3), a technique that is gaining popularity in genotyping and heteroduplex detection following PCR.¹ Dye redistribution, which is caused by low dye concentration, is an unsolvable problem for SYBR® Green I because its high tendency to inhibit PCR requires that the dye be used at a very low concentration. Thus, SYBR® Green I is not suitable for either multiplex PCR or HRM.^{1,2} Moreover, dye redistribution may make SYBR® Green I unreliable even for regular melt curve analysis because low-melting DNA species may simply not have been detected.^{2,3}

Second, EvaGreen™ is an extremely stable dye. Under the normal conditions of storage, handling and PCR operation, the dye is virtually indestructible. The dye in a buffer can be safely stored at room temperature or in a refrigerator, or can be subject to multiple freeze-thaw cycles. On the other

hand, SYBR® Green I is known to be unstable and the decomposed dye is even more inhibitory to PCR.⁴

Third, we have made EvaGreen™ a much safer dye than SYBR® Green I by rendering it cell membrane-impermeable (Figure 5). Indeed, an independent laboratory test has shown that EvaGreen™ is both nonmutagenic and noncytotoxic.⁵ In comparison, although SYBR® Green I is only weakly mutagenic by itself, it has been shown to be a potent mutation-enhancer by possibly inhibiting the natural DNA repairing mechanism in cells.⁶ In view of the widespread practice of qPCR, we believe that safety of the dye should be an important consideration.

Four EvaGreen™ products are offered. EvaGreen™ 20X (#31000) is a solution containing optimally adjusted dye concentration that can be diluted 20 times for qPCR use. EvaGreen™ 25 mM in DMSO (#31002) is a highly concentrated dye solution for researchers who have become familiar with the dye and wish to develop their own qPCR formulations or to explore other applications with the dye. EvaGreen™ Master Mix (#31003) contains everything you need except for the primer set to amplify your target DNA, while EvaGreen™ Basic Mix (#31001) is similar to the master mix except that no enzyme is included.

References:

- 1) Herrmann, et al. *Clin. Chem.* **52**(3), 494(2006).
- 2) Wittwer, et al. *Clin. Chem.* **49**(6), 853(2003).
- 3) Giglio, et al. *Nucleic acids Res.* **31**(22), e136(2003).
- 4) Karsai, et al. *BioTechniques* **32**(4), 790(2002).
- 5) A full safety report is available at Biotium website.
- 6) Ohta et al. *Mut. Res.* **492**, 91(2001).

EvaGreen™ for qPCR

EvaGreen™ Is Compatible with Fast PCR Protocol

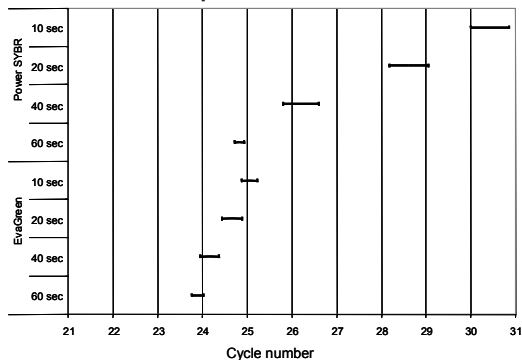


Figure 2. Comparison of EvaGreen™ Master Mix and Power SYBR® Master Mix (ABI) in GAPDH amplification. The two-stage Universal Cycling condition was used with 15 seconds at 95 °C and a varying amount of annealing/extension time (60, 40, 20 and 10 seconds, respectively) at 60 °C. Each horizontal bar represents the Ct distribution of 8 repeat experiments. The data shows that reduction in annealing/extension time from 60 s to 10 s resulted in only about one cycle delay in Ct with EvaGreen Master Mix, but as many as 5-6 cycle delay in Ct and poor reproducibility with Power SYBR Master Mix.

EvaGreen™ Can Be Used for High-resolution Melt Curve Analysis

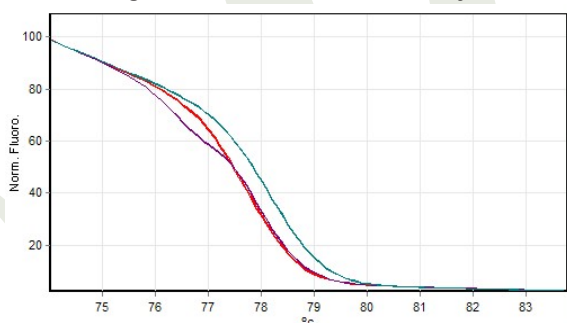


Figure 3. High-resolution DNA melt curve (HRM) analysis using EvaGreen™ on Rotor Gene 6000 clearly distinguishes three different genotypes: mutant (red), heterozygous (purple) and wild (blue). (Data contributed by Corbett Research).

Table 1. EvaGreen™ Product List

Cat No.	Product Name	Unit Size
31000	EvaGreen™ 20X in water	5 x 1 ml
31001	EvaGreen™ qPCR 2X Basic Mix for non hotstart Taq	3 x 1.7 ml
31002	EvaGreen™ 25 mM in DMSO	1ml
31004	EvaGreen™ qPCR 2X Basic Mix for hotstart Taq	3 x 1,7 ml available soon

* For price details please see our special promotion under "Special Offers"

EvaGreen™ Can Be Optimally Excited by the 488 Ar Laser or Blue LED

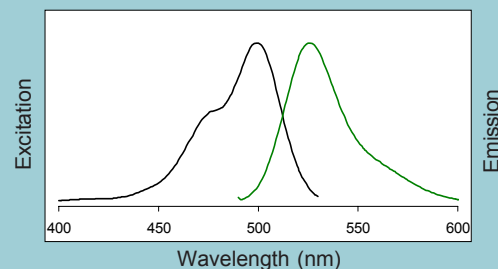
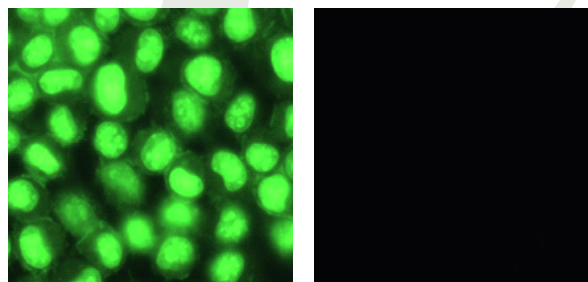


Figure 4. Excitation and emission spectra of EvaGreen™ in the presence of dsDNA in PBS buffer.

EvaGreen™ Is Safer than SYBR® Green I by Being Impermeable to Cell Membranes



1.2 μM SYBR® Green I

1.2 μM EvaGreen™

Figure 5. HeLa Cells incubated with either SYBR® Green I (1.2 μM, left panel) or EvaGreen™ (1.2 μM, right panel) at 37 °C were followed by fluorescence microscopy. No cell staining was observed with EvaGreen™ during 30 minutes of incubation, suggesting that EvaGreen™ did not cross cell membranes. However, significant cellular staining was observed with SYBR® Green I in less than 5 minutes of incubation (data not shown). After 30 minutes, SYBR® Green I stained cell nuclei intensely green (left panel). The rapid cellular uptake of SYBR® Green I, coupled with the dye's known potent mutation-enhancing ability (Ohta et al. *Mutation Research* **492**, 91(2001)), makes the dye a potential hazard during routine PCR operation. EvaGreen™, on the other hand, appears to be completely membrane-impermeable, which may be at least partially responsible for its nonmutagenicity and noncytotoxicity as confirmed by standard Ames test (A full safety report on EvaGreen™ can be downloaded at Biotium website).

EvaGreen™ is for end-users only. To license the technology for commercial use, please contact Biotium. To practice qPCR and/or HRM, you may need additional licenses from ABI, Roche and any other relevant patent holders.

Another breakthrough qPCR technology, AllGlo™ Probes, may also be licensed through Biotium. Developed by Allelogic Biosciences Corp, AllGlo™ Probes are superior alternatives to TaqMan®-MGB probes by having better discrimination power in detecting single mutation sites, better signal strength and much simpler design.