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**

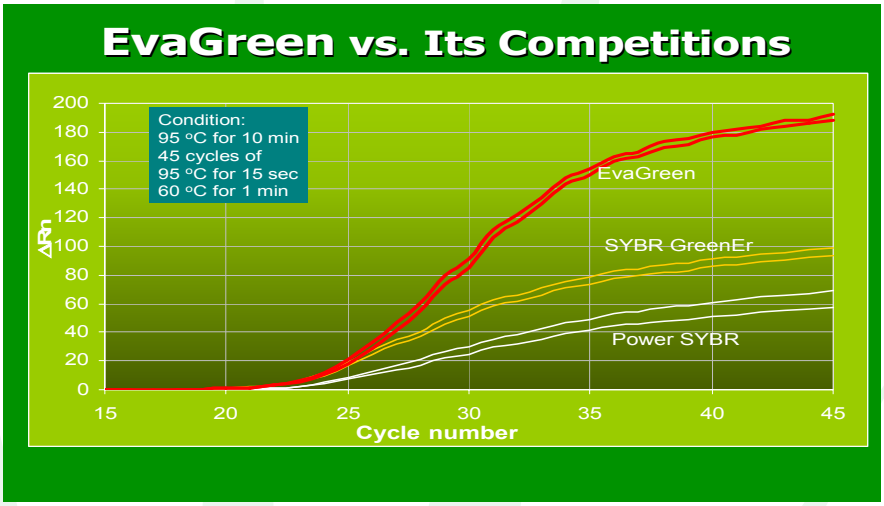


Figure 1. Amplifications of a Myc gene fragment (75 ng huDNA input) (ABI) using EvaGreen™ (red), SYBR® GreenER™ (green) (Invitrogen) and Power SYBR® (yellow) (ABI), respectively. All reactions were run in duplicate on an ABI 7900 instrument using the Universal cycling protocol (15 s at 95 °C and 60 s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

We offer several EvaGreen® products. EvaGreen® Master Mix (#31003) contains everything you need except for the primer set to amplify your target DNA. EvaGreen® Master Mix is also suitable for high resolution melt curvem(HRM) analysis. EvaGreen® 20X (#31000) is a solution containing optimally adjusted dye concentration that can be diluted 20 times to make your own EvaGreen® master mix.

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).

Biotium

Glowing Products for Science™

EvaGreen™ for qPCR

EvaGreen™ Is Compatible with Fast PCR Protocol

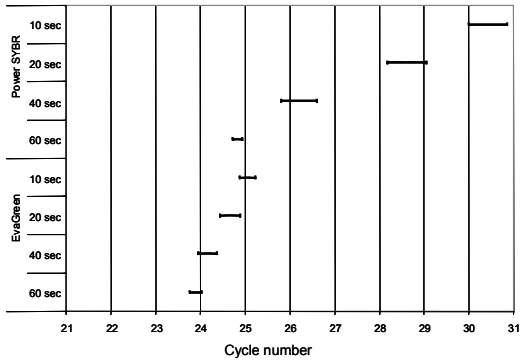


Figure 2. Comparison of EvaGreen™ Master Mix and Power SYBR® Master Mix (ABI) in GAPDH amplification. The two-stage Universal Cycling condition was used with 15 seconds at 95 °C and a varying amount of annealing/extension time (60, 40, 20 and 10 seconds, respectively) at 60 °C. Each horizontal bar represents 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

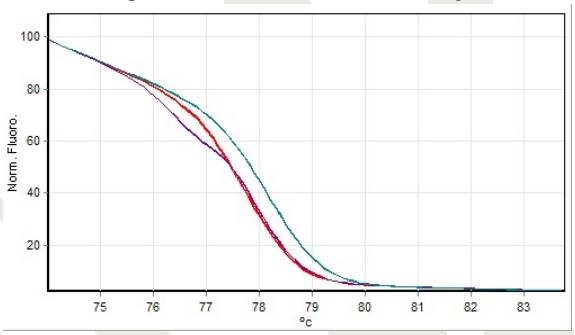


Figure 3. High-resolution DNA melt curve (HRM) analysis using EvaGreen™ on Rotor Gene 6000 clearly distinguishes three different genotypes: mutant (red), heterozygous (purple) and wild (blue). (Data contributed by Corbett Research).

Table 1. EvaGreen® Product List

Cat.#	Product Name	Unit Size	Unit Price ($)
31003	*Fast* EvaGreen® Master Mix for qPCR and HRM	2x1 mL	120.00
31003-1	*Fast* EvaGreen® Master Mix for qPCR and HRM	5x1 mL	275.00
31003-2	*Fast* EvaGreen® Master Mix for qPCR and HRM	50x1 mL	2500.00
31000	EvaGreen® dye, 20x in water	5x1 mL	150.00
31001	EvaGreen® qPCR 2X Basic Mix	3x1.7 mL	150.00
31004	EvaGreen® qPCR 2X Basic Mix for Hot Start Taq	3x1.7 mL	200.00

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

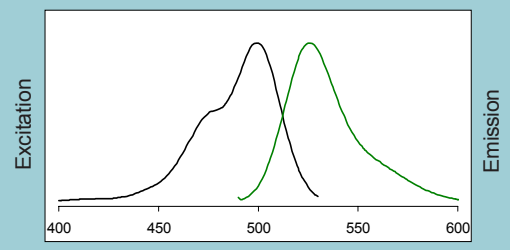
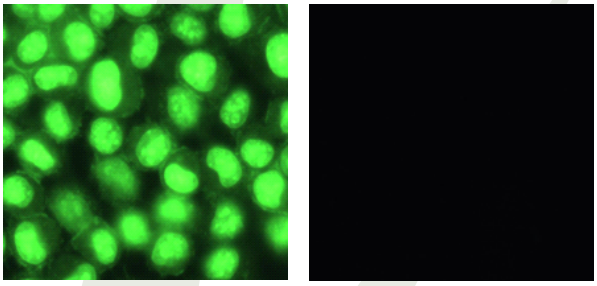


Figure 4. Excitation and emission spectra of EvaGreen™ in the presence of dsDNA in PBS buffer.

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.