**EvaGreen**

**The very best dye for qPCR and HRM**

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**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**

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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. 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.2,3 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.

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**References:**

5) A full safety report is available at Biotium website.

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

**EvaGreen™**

- **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.

- **Figure 3.** High-resolution 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

<table>
<thead>
<tr>
<th>Cat.#</th>
<th>Product Name</th>
<th>Unit Size</th>
<th>Unit Price ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>31000</td>
<td>EvaGreen™ 20X in water</td>
<td>5x1 mL</td>
<td>150.00</td>
</tr>
<tr>
<td>31001</td>
<td>EvaGreen™ qPCR 2X Basic Mix</td>
<td>3x1.7 mL</td>
<td>150.00</td>
</tr>
<tr>
<td>31002</td>
<td>EvaGreen™, 25 mM in DMSO</td>
<td>1 mL</td>
<td>400.00</td>
</tr>
<tr>
<td>31003</td>
<td>EvaGreen™ qPCR 2X Master Mix</td>
<td>2x1 mL (200 reaction)</td>
<td>coming soon</td>
</tr>
</tbody>
</table>

- **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.

- **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).