

# Product Information

## EvaGreen® Dye, 20X in water

**Catalog Number:** 31000-T, 31000

### Size

31000-T: 1 mL  
31000: 5 x 1 mL

**Concentration:** 20X (25  $\mu$ M) in water

**Color and Form:** Light orange solution

### Spectral Properties

$\lambda_{\text{abs}}$  = 471 nm (without DNA)  
 $\lambda_{\text{abs}}/\lambda_{\text{em}}$  = 500/530 nm (with DNA)

### Storage and Handling

Store at  $-20^{\circ}\text{C}$ , protected from light. EvaGreen® Dye is very stable and may also be stored at  $4^{\circ}\text{C}$ , however, because this product is preservative-free we recommend storing at  $-20^{\circ}\text{C}$  to avoid contamination.

### Product Description

EvaGreen® dye is a green fluorescent nucleic acid dye with features that make the dye useful for several applications including qPCR<sup>1,2</sup>, melt curve analysis<sup>3</sup>, real-time monitoring of thermophilic helicase-dependent amplification (tHDA)<sup>4</sup>, routine solution DNA quantification<sup>5,6</sup> and capillary gel electrophoresis<sup>7,8</sup>. The DNA-bound dye has excitation and emission spectra very close to those of fluorescein (FAM) or SYBR® Green I (Figure 1), making the dye readily compatible with instruments equipped with the 488 nm argon laser or any visible light excitation with wavelength in the region. EvaGreen dye is extremely stable both thermally and hydrolytically (Figure 2), providing convenience during routine handling. The dye is essentially non-fluorescent by itself, but becomes highly fluorescent upon binding to dsDNA. EvaGreen dye is nonmutagenic and noncytotoxic because it is impermeable to cell membranes (Figure 3), unlike SYBR Green I, which enters cell rapidly and is known to be a powerful mutation-enhancer<sup>9</sup>.

The unique properties of EvaGreen dye have made it particularly useful in quantitative real-time PCR (qPCR) application. Compared with the widely used SYBR Green I, EvaGreen dye is generally less inhibitory toward PCR and less likely to cause nonspecific amplification. As a result, EvaGreen dye can be used at a much higher dye concentration than SYBR Green I, resulting in more robust PCR signal. More significantly, the higher EvaGreen concentration permitted for qPCR eliminates "dye redistribution" problems, which can occur with SYBR Green I during post-PCR DNA melt curve analysis. Dye redistribution problems may make SYBR Green I unreliable for DNA melt curve analysis (Giglio, et al. *Nucleic Acid Res.* 31(22), e136(2003); Wittwer, et al. *Clin. Chem.* 49(6), 853(2003)). On the other hand, EvaGreen dye is optimal for both qPCR and melt curve analysis, yielding robust and reproducible results.

EvaGreen 20X in water is specifically formulated for qPCR use. The PCR reaction can be monitored using your existing optical setting for SYBR Green I or FAM on any commercial real-time PCR cyclor. The qPCR protocol provided below is for PCR using regular non-hot-start Taq. Use of a hot-start Taq may require some adjustment of PCR buffer composition in terms of ionic strength and pH to best take the advantage of EvaGreen dye. For example, chemically-modified Taq, such as AmpliTaq Gold, may prefer a lower concentration of KCl or no KCl and higher Tris concentration (up to 50 mM). In addition, a water soluble solvent such as DMSO or glycerol are frequently added to stabilize master mixes. These components and pH may need to be optimized depending on the enzyme used.

Using non-hot-start Taq and the protocol provided below, EvaGreen dye shows higher fluorescent signals than SYBR Green I for both qPCR and melt curve analysis. Because the optical settings vary slightly from instrument to instrument and the wavelengths of EvaGreen dye are slightly longer than those of SYBR Green I, Ct value may differ slightly by +1 or -1 when compared with SYBR Green I side-by-side.

## Protocol

The following protocol is recommended for use with non-hotstart Taq.

1. Set up the PCR reaction as follows<sup>1</sup>:  
5  $\mu$ L of 10x polymerase buffer without magnesium<sup>2</sup>  
2.5  $\mu$ L of 50mM  $\text{MgCl}_2$ <sup>3</sup>  
5  $\mu$ L each of 2 mM dNTP  
2.5  $\mu$ L of 20X EvaGreen<sup>4</sup>  
1-5 units of Taq DNA polymerase<sup>5</sup>  
0.1-1  $\mu$ M each of primers (final concentrations)  
 $\text{dH}_2\text{O}$  to a final volume of 50  $\mu$ L.

2. Perform real-time PCR on a thermocycling fluorometer and record the fluorescence signal at the annealing or extension step.

Note: When using ABI Sequence Detection Systems, make sure to select NONE for the passive reference under the tab WELL INSPECTOR.

Note: BSA may be required if the reaction is run on a Roche LightCycler. A final BSA concentration of 0.5 mg/mL may be sufficient. With SYBR Green, addition of a protein such as BSA results in a fluorescence increase, which provides a background signal that triggers the start of a LightCycler. Because EvaGreen dye is less sensitive to proteins, you may need to adjust the instrument setting (for background fluorescence) so that the instrument will start.

- <sup>1</sup> For iCycler users, you do not need to add FAM to your PCR mix because EvaGreen has a slight background fluorescence that provides an adequate and stable baseline level fluorescence for well calibration.
- <sup>2</sup> For chemically-modified Taq, it may be necessary to reduce the KCl concentration and increase the Tris concentration.
- <sup>3</sup> The optimal  $\text{Mg}^{2+}$  concentration for PCR with EvaGreen dye is 2.5 mM.
- <sup>4</sup> Before pipetting, warm up the 20X solution to room temperature and thoroughly mix the solution by vortexing. EvaGreen is highly stable. However, dye may adsorption to the vial during storage. Vortex the vial for a few seconds to make sure the dye is fully dissolved.
- <sup>5</sup> For best results, a hot-start enzyme should be used. However, buffer formulation may need to be adjusted.

## Safety

Ames testing performed by an independent lab, Litron Laboratories (Rochester, NY), showed that EvaGreen dye is nonmutagenic as well as non-cytotoxic. EvaGreen dye appears to be completely cell membrane-impermeable (Figure 3), which may be a key factor responsible for the observed low toxicity. On the other hand, SYBR Green I is known to be a powerful mutation enhancer, possibly by inhibiting the natural DNA repairing mechanism in cells (Ohta, et al. *Mutat. Res.* 492, 91(2001)). The toxicity of SYBR Green I may be associated with its ability to enter cells rapidly (Figure 3). You can download a complete safety report on EvaGreen dye at [www.biotium.com](http://www.biotium.com). Although EvaGreen has undergone extensive safety testing, we advise researchers to exercise universal laboratory safety precautions when handling EvaGreen dye or any other DNA-binding agents.

## Disposal

EvaGreen dye at 2X is classified as nonhazardous for drain disposal under CCR Title 22 regulation. If required by your local regulations, EvaGreen can be adsorbed onto activated charcoal for disposal. Pour 10 liters of EvaGreen waste solution through ~1g of activated charcoal. The filtrate may directly go to the drain while the charcoal may be treated as solid waste.

## References

1. Mao, et al. Characterization of EvaGreen Dye and the implication of its physicochemical properties for qPCR applications. *BMC Biotechnology* 7, 76 (2007).
2. Novak, et al. An integrated fluorescence detection system for lab-on-a-chip applications. *Lab Chip* 7, 27(2007).
3. White, et al. Methylation-sensitive high-resolution melt-curve analysis of the SNRPN gene as a diagnostic screen for Prader-Willi and Angelman Syndromes. *Clin. Chem.* 53(11), 1 (2007).
4. Goldmeyer, et al. Development of a novel one-tube isothermal reverse transcription thermophilic helicase-dependent amplification platform for rapid RNA detection. *J. Mol. Diag.* 9(5), 639 (2007).
5. Wang, et al. DNA quantification using EvaGreen and a real-time PCR instrument. *Anal. Biochem.* 356, 303 (2006).
6. Ihrig, et al. Application of the DNA-specific dye EvaGreen for the routine quantification of DNA in microplates. *Anal. Biochem.* 359, 265 (2006).
7. Sang, et al. Genetic mutation analysis by CE with LIF detection using inverse-flow derivatization of DNA fragments. *Electrophoresis* 27, 3846 (2006).
8. Sang, et al. Capillary electrophoresis of double-stranded DNA fragments using a new fluorescence intercalating dye EvaGreen. *J. Sep. Sci.* 29, 1275 (2006).
9. Ohta, et al. Ethidium bromide and SYBR Green I enhance the genotoxicity of UV-irradiation and chemical mutagens in *E. coli*. *Mutat. Res.* 492, 91 (2001).

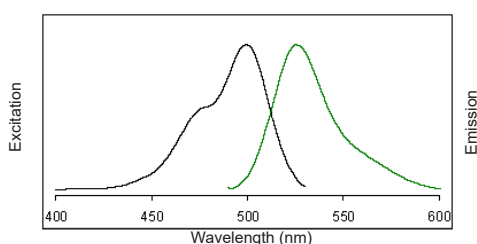


Figure 1. Excitation (left) and emission (right) spectra of EvaGreen™ dye bound to dsDNA in pH 7.3 PBS buffer.

## Related Products

Cat. No.	Product Name
31019	EvaGreen® Dye, 2000X in DMSO, 50 uL
29050	Cheetah™ HotStart Taq DNA Polymerase, 500 U
29052	ROX reference dye, 25 uM in TE buffer, 5 X 1 mL
31003	Fast EvaGreen® qPCR Master Mix, 200 reactions
31020	Fast Plus EvaGreen® qPCR Master Mix, No Rox, 200 reactions
31014	Fast Plus EvaGreen® qPCR Master Mix, Low Rox, 200 reactions
31015	Fast Plus EvaGreen® qPCR Master Mix, High Rox, 200 reactions
31005	Fast Probe qPCR Master Mix, No Rox, 200 reactions
31016	Fast Probe qPCR Master Mix with Rox, 200 reactions
41001	GelRed® Nucleic Acid Gel Stain, 3X in H <sub>2</sub> O, 4L
41003	GelRed® Nucleic Acid Gel Stain, 10,000X in H <sub>2</sub> O, 0.5 mL
41005	GelGreen® Nucleic Acid Gel Stain, 10,000X in H <sub>2</sub> O, 0.5 mL
31030	DNA Gel Extraction Kit, 50 or 250 columns
31021	1 KB DNA Ladder (100 ng/uL), 30 ug/300 ul
31022	Ready-to-Use 1 KB DNA Ladder, 150 applications (1.5 ml)
31031	100 bp DNA Ladder (100 ng/uL), 30 ug/300 ul
31032	Ready-to-Use 100 bp DNA Ladder, 150 applications (1.5 ml)
41006	TBE Buffer, 5X, 4L

Please visit our website at [www.biotium.com](http://www.biotium.com) for information on our life science research products, including AccuBlue™ and AccuClear™ DNA quantitation kits, Lumitein™ fluorescent gel stain, fluorescent CF™ dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.

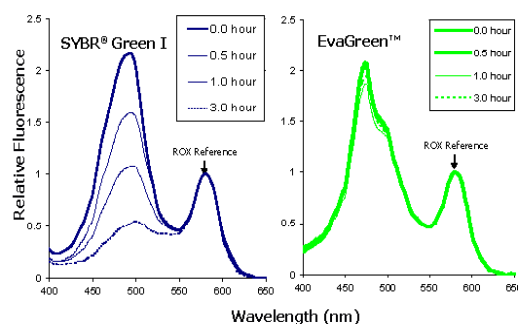


Figure 2. Stability comparison of EvaGreen and SYBR Green dyes. Solutions of EvaGreen dye or SYBR Green I at 1.2  $\mu$ M in pH 9 Tris buffer were incubated at 99 °C. The absorption spectrum of each solution was measured over a period of 3 hours. ROX was added as a stable reference.

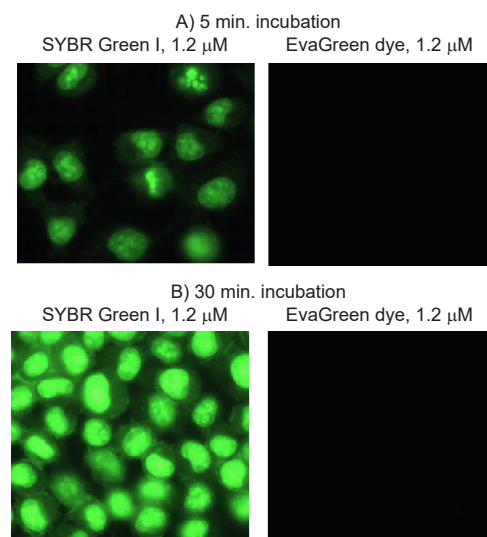


Figure 3. Cell permeability of EvaGreen and SYBR Green dyes. HeLa cells were incubated with SYBR Green I (1.2  $\mu$ M) or EvaGreen dye (1.2  $\mu$ M) at 37 °C. Images were taken following incubation for 5 min (panel A) and 30 min (panel B). SYBR Green I entered cells rapidly while EvaGreen was membrane-impermeable.

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Practicing real-time PCR may require additional licensing from Roche or Applied Biosystems. Practicing high-resolution melt curve analysis may require additional licensing from Idaho Technologies.