

Product Information

EvaGreen® Dye, 20X in Water

Catalog Number: 31000-T, 31000

Unit Size

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

Concentration: 20X (25 µM) in water

Color and Form: Light orange solution

Spectral Properties

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

Storage and Handling

Store at 4°C, protected from light. EvaGreen® Dye is extremely stable both thermally and chemically (1). However, because this product is preservative-free, after opening we recommend storing in aliquots at -20°C to avoid contamination.

Product Description

EvaGreen® Dye is a green fluorescent nucleic acid dye with features that are ideal for a wide variety of applications including qPCR (2,3), DNA melt curve analysis (4), HRM®, LAMP, digital PCR, real-time monitoring of thermophilic helicase-dependent amplification (tHDA) (5), DNA quantification (6,7), and capillary gel electrophoresis (8,9). The dye is essentially non-fluorescent by itself, but becomes highly fluorescent upon binding to dsDNA. The DNA-bound dye has excitation and emission spectra (Fig. 1) that are very close to those of fluorescein (FAM) or SYBR® Green I, 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 (1), providing convenience during routine handling. In addition, the dye is non-mutagenic and non-cytotoxic because it is cell membrane-impermeant, unlike SYBR® Green I, which enters cells rapidly and is known to be a powerful mutation enhancer (10).

The unique properties of EvaGreen® Dye have made it particularly useful in quantitative real-time PCR (qPCR). 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 (Fig. 2). More significantly, the higher EvaGreen® Dye concentration permitted for qPCR eliminates problems caused by dye redistribution that make SYBR® Green I unreliable for high resolution DNA melt curve analysis (11,12). Consequently, EvaGreen® Dye is optimal for both qPCR and HRM® analysis, yielding robust and reproducible results.

EvaGreen® Dye 20X in Water is a convenient concentration 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 cyclers. An example protocol provided is for qPCR using Biotium's Cheetah™ HotStart Taq; qPCR conditions may require optimization for specific targets or sample types.

We also offer EvaGreen® Dye in a 2000X concentration in DMSO for when higher concentrations are needed, as well as optimized Forget-Me-Not™ Master Mixes that include EvaGreen® Dye. Also see our EvaGreen® Plus Dye, which has higher signal and lower background compared to EvaGreen® Dye (see Related Products).

General Considerations

- Before use, warm up the 20X solution to room temperature and thoroughly mix the solution by vortexing, dye may adhere to the vial during storage.
- 1X concentration is recommended for qPCR. For other applications, it is recommended to titrate dye up to 2X concentration or higher.
- The optimal Mg²⁺ concentration for PCR with EvaGreen® Dye is 2.5 mM.
- EvaGreen® Dye can be used for high resolution melting (HRM®) analysis. Follow your qPCR system's instructions for data collection and analysis.
- If you are using ABI Sequence Detection Systems, make sure to select NONE for the passive reference under the tab WELL INSPECTOR.
- For iCycler® users, you do not need to add FAM to your PCR mix because EvaGreen® Dye has a slight background fluorescence that provides an adequate and stable baseline level fluorescence for well calibration.
- 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.

Protocol for qPCR

The following is an example protocol for qPCR using Biotium's Cheetah™ HotStart Taq. Reaction conditions may require optimization for different applications.

1. Set up PCR reaction using the following final concentrations of reaction components:
 - 1X Cheetah™ Taq Polymerase Buffer
 - 2.5 mM MgCl₂
 - 0.1-1 µM each of primers
 - 0.2 mM each of dNTPs
 - 0.02-0.1 unit/µL Cheetah™ HotStart Taq DNA Polymerase
 - 1X EvaGreen® Dye
 - Optional ROX Reference Dye (if required by your instrument)
 - dH₂O to required final reaction volume
2. Perform real-time PCR on a qPCR instrument and acquire the fluorescence signal at the annealing or extension step with the SYBR® Green or FAM channel.
3. After PCR with EvaGreen® Dye, PCR products do not need to be stained with another DNA gel stain for gel electrophoresis. Simply add DNA loading buffer to your PCR reaction solution, load on a gel, and conduct electrophoresis as usual. Gel visualization can be carried out using a 254 nm UV box, or a blue LED imager using a SYBR® Green filter. Alternatively, the gel may be imaged using a 488 nm laser-based gel scanner.

Safety

Ames testing performed by an independent lab, Litron Laboratories (Rochester, NY), showed that EvaGreen® Dye is non-mutagenic as well as non-cytotoxic. EvaGreen® Dye appears to be completely cell membrane-impermeant, 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 (10). The toxicity of SYBR® Green I may be associated with its ability to enter cells rapidly. You can download a complete safety report on EvaGreen® Dye at www.biotium.com. Although EvaGreen® Dye has undergone extensive safety testing, we advise researchers to exercise universal laboratory safety precautions when handling EvaGreen® Dye or any other DNA-binding agents.

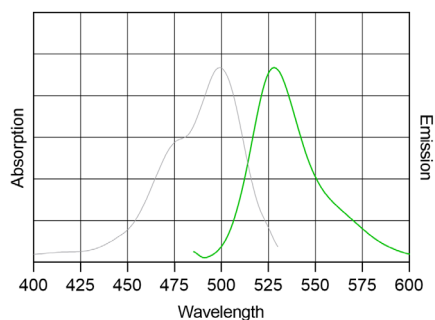


Figure 1. Excitation (left) and emission (right) spectra of EvaGreen® Dye bound to dsDNA in TBE buffer.

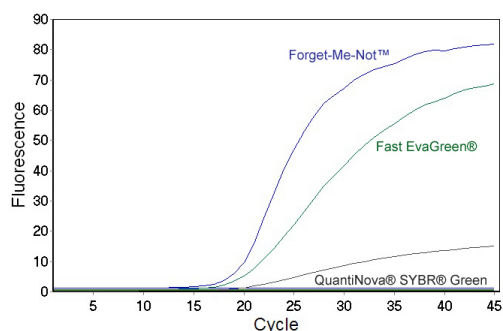


Figure 2. A comparison of the raw fluorescence signal from qPCR reactions performed with two EvaGreen® master mixes (Forget-Me-Not™ EvaGreen® and Fast EvaGreen®) and QuantiNova® SYBR® Green. EvaGreen® Dye is less inhibitory than SYBR® green, allowing for a much brighter signal.

Disposal

EvaGreen® Dye at 2X is classified as nonhazardous for drain disposal under CCR Title 22 regulation. If required by your local regulations, EvaGreen® Dye can be removed from solutions using Biotium's Activated Charcoal Decontamination Bags (see Related Products). Alternatively, pour 10 liters of EvaGreen® Dye 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

EvaGreen® Dye has been validated in thousands of peer-reviewed publications. Visit www.biotium.com to download an a list of selected references for various applications.

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- Novak, et al. An integrated fluorescence detection system for lab-on-a-chip applications. *Lab Chip* 7, 27 (2007).
- 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).
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- Sang, et al. Genetic mutation analysis by CE with LIF detection using inverse-flow derivatization of DNA fragments. *Electrophoresis* 27, 3846 (2006).
- Sang, et al. Capillary electrophoresis of double-stranded DNA fragments using a new fluorescence intercalating dye EvaGreen. *J. Sep. Sci.* 29, 1275 (2006).
- 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).

- Wittwer, et al. High-resolution genotyping by amplicon melting analysis using LCGreen. *Clin. Chem.* 49, 853(2003).
- Giglio, et al. Demonstration of preferential binding of SYBR Green I to specific DNA fragments in real-time multiplex PCR. *Nucleic Acids Res.* 31, (2003).

Related Products

Catalog number	Product
31019	EvaGreen® Dye, 2000X in DMSO
31077	EvaGreen® Plus Dye, 20X in Water
29050	Cheetah™ HotStart Taq DNA Polymerase, 500 U
29052	ROX reference dye, 25 uM in TE buffer
31045, 31046	Forget-Me-Not™ EvaGreen® qPCR Master Mix
31041, 31042	Forget-Me-Not™ EvaGreen® qPCR Master Mix, (2-Color Tracking)
41001	GelRed® Nucleic Acid Gel Stain, 3X in H ₂ O
41003	GelRed® Nucleic Acid Gel Stain, 10,000X in H ₂ O
41005	GelGreen® Nucleic Acid Gel Stain, 10,000X in H ₂ O
41011	GelRed® Prestain Plus 6X DNA Loading Dye
41010	6X GelRed® Prestain Loading Buffer, Orange Tracking Dye
41029	GelRed® Agarose LE
41030	GelGreen® Agarose LE
41008, 41014	PAGE GelRed® Nucleic Acid Gel Stain
41024-4L	Water, Ultrapure Molecular Biology Grade (4L Cubitainer®)
31030	DNA Gel Extraction Kit
31022	Ready-to-Use 1 KB DNA Ladder
31032	Ready-to-Use 100 bp DNA Ladder
41006	TBE Buffer, 5X
22007	Activated Charcoal Decontamination Bags

Please visit our website at www.biotium.com for information on our life science research products, including AccuBlue® and AccuClear® DNA quantitation kits, One-Step protein gel stains, fluorescent CF® Dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.

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.

EvaGreen and applications are covered under granted and pending US and international patents. SYBR is a registered trademark of Invitrogen, Inc, HRM is a registered trademark of Idaho Technologies; iCycler is a registered trademark of Bio-Rad; LightCycler is a registered trademark of Roche; QuantiNova is a registered trademark of Qiagen; SYBR is a registered trademark of Thermo Fisher Scientific.

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