Revised: October 19, 2022

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 uM) in water

Color and Form: Light orange solution

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

Storage and Handling

EvaGreen® Dye is extremely stable both thermally and chemically (1). We recommend storing the unopened stock solution at room temperature, protected from light. EvaGreen® Dye stock solution can also be stored at 4°C or -20°C without affecting performance. After opening, we recommend storing this preservative-free dye in aliquots at -20°C to prevent microbial growth. When stored as recommended, EvaGreen® Dye is stable for at least 12 months from the date of receipt.

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), DNA melt curve analysis, HRM®, LAMP, digital PCR, real-time monitoring of thermophilic helicase-dependent amplification (tHDA), DNA quantification, and capillary gel electrophoresis. See the EvaGreen® Dye reference list for selected references by application.

EvaGreen® Dye is essentially non-fluorescent by itself, but becomes highly fluorescent upon binding to dsDNA. EvaGreen® Dye can be excited by instruments with a 488 nm laser or light excitation of a similar wavelength. The EvaGreen® excitation and emission spectra (Fig. 1) are very similar to those of FAM or SYBR®, making the dye readily compatible for use with instruments with those detection channels. 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 (3).

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 non-specific 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 (4,5). 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 cycler. An example protocol using Biotium's Cheetah™ HotStart Taq for qPCR is provided; qPCR conditions may require optimization for specific targets or sample types.

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

Considerations for Use

- 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 Mg^{2+} 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.
- When using Applied Biosystems® 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 $MgCl_2$
   0.1-1 uM each of primers
   0.2 mM each of dNTPs
   0.02-0.1 unit/uL Cheetah™ HotStart Taq DNA Polymerase
   1X EvaGreen® Dye
   Optional ROX Reference Dye (if required by your instrument)
   dH_2O 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 can be analyzed by gel electrophoresis without the need for an additional gel stain. Simply add DNA loading buffer to your PCR reaction solution, load on a gel, and perform 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 (3). The toxicity of SYBR® Green I may be associated with its ability to enter cells rapidly. A complete EvaGreen® Safety Report can be downloaded from the Bioium website. 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.

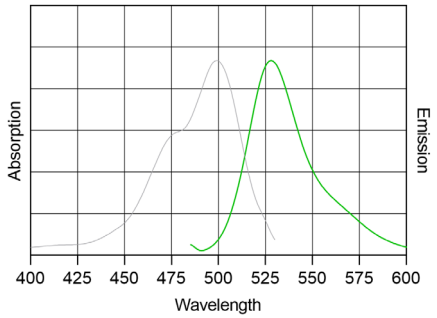

Figure 1. Excitation (left) and emission (right) spectra of EvaGreen® Dye bound to dsDNA in TBE buffer.

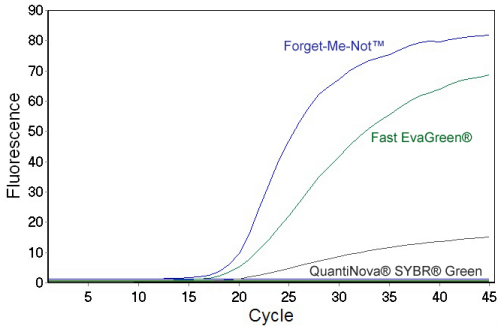

Figure 2. A comparison of the raw fluorescence signal from qPCR reactions performed with two EvaGreen® master mixes (Forget-Me-Not™ EvaGreen® and Fast EvaGreen®) and QuantiNova® SYBR® Green. EvaGreen® Dye is less inhibitory than SYBR® Green, allowing for a much brighter signal.

Disposal

EvaGreen® Dye at concentrations of 2X and below are classified as non-hazardous 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 up to 10 liters of 1X EvaGreen® Dye waste solution through ~1 g of activated charcoal. The filtrate may be disposed of directly in the drain while the charcoal may be treated as solid waste.

References

EvaGreen® Dye has been validated in thousands of peer-reviewed publications. View our full list of EvaGreen® references for various applications.

1. Nowadly, et al. Characterization of the Effects of Heat Stress on the DNA-Intercalating Dye EvaGreen for Potential Use With the Joint Biological Agent Identification and Diagnostic System. Mil Med 179(6), 626 (2014).
2. Mao, et al. Characterization of EvaGreen® Dye and the implication of its physicochemical properties for qPCR applications. BMC Biotechnol. 7, 76 (2007).
3. Ohta, et el. Ethidium bromide and SYBR® Green I enhance the genotoxicity of UV-irradiation and chemical mutagens in E. coli. Mutat. Res. 492, 91 (2001).
4. Wittwer, et al. High-resolution genotyping by amplicon melting analysis using LCGreen. Clin. Chem, 49, 853(2003).
5. 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

Cat. No.	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
31079	EvaRuby™ Dye, 20X in Water
29087	VeriFluor™ Far-Red Passive Reference Dye, 400X in Water
31045, 31046	Forget-Me-Not™ EvaGreen® qPCR Master Mix (Low ROX or High ROX)
31041, 31042	Forget-Me-Not™ EvaGreen® qPCR Master Mix, (2-Color Tracking)
41001	GelRed® Nucleic Acid Gel Stain, 3X in H_2O
41003	GelRed® Nucleic Acid Gel Stain, 10,000X in H_2O
41005	GelGreen® Nucleic Acid Gel Stain, 10,000X in H_2O
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.

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