Revised: April 27, 2021

Product Information

EvaGreen® Dye, 2000X in DMSO

Catalog Number: 31019

Unit Size: 50 uL

Concentration: 2000X (2.5 mM) in DMSO

Color and Form: Orange solution

Spectral Properties

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

Storage and Handling

Store at room temperature or 4°C protected from light. Product is stable for at least 12 months from the date of receipt when stored as recommended. The solution may also be stored at -20°C without affecting its performance. However, under cold storage conditions, dye precipitation may occur, in which case the vial containing the dye may be heated to 60°C with occasional vortexing for one hour, or until the dye redissolves. You can confirm that the dye concentration is accurate after redissolving by measuring the dye absorbance using a spectrophotometer. When diluted 1:100 in 1X PBS buffer (pH 7.4), the absorbance of the dye solution at 469 nm should be 2 +/- 0.15. For convenience, the 2.5 mM concentrated solution may be diluted 10 times to a 0.25 mM solution in either dH_2O or Tris (10 mM, pH 7-9), which may be stored at 4°C.

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, 2000X in DMSO is a highly concentrated solution for customers with specialized applications. An example protocol provided is for a typical qPCR reaction using Biotium's Cheetah™ HotStart Taq; qPCR conditions may require optimization for specific targets or sample types. The PCR reaction can be monitored using your existing optical setting for SYBR® Green I or FAM on any commercial real-time PCR cycler.

We also offer EvaGreen® Dye in a 20X concentration in water, 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 2000X 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.
- 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 $MgCl_2$
   - 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 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.

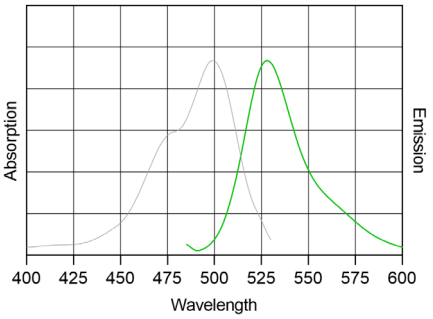

Figure 1. Excitation (left) and emission (right) spectra of EvaGreen® Dye bound to dsDNA in TBE buffer.

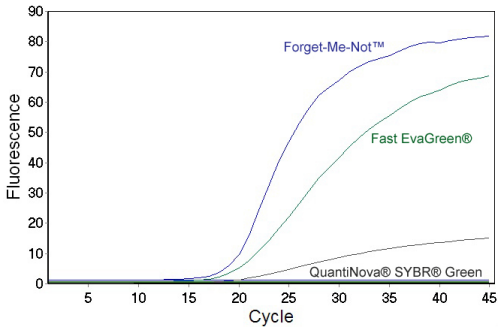

Figure 2. A comparison of the raw fluorescence signal from qPCR reactions performed with two EvaGreen® master mixes (Forget-Me-Not™ EvaGreen® and Fast EvaGreen®) and QuantiNova® SYBR® Green. EvaGreen® Dye is less inhibitory than SYBR® green, allowing for a much brighter signal.

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.

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 Biotechnology 7, 76 (2007).
3. Novak, et al. An integrated fluorescence detection system for lab-on-a-chip applications. Lab Chip 7, 27 (2007).
4. 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).
5. 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).
6. Wang, et al. DNA quantification using EvaGreen and a real-time PCR instrument. Anal. Biochem. 356, 303 (2006).
7. Ihrig, et al. Application of the DNA-specific dye EvaGreen for the routine quantification of DNA in microplates. Anal. Biochem. 359, 265 (2006).
8. Sang, et al. Genetic mutation analysis by CE with LIF detection using inverse-flow derivatization of DNA fragments. Electrophoresis 27, 3846 (2006).
9. Sang, et al. Capillary electrophoresis of double-stranded DNA fragments using a new fluorescence intercalating dye EvaGreen. J. Sep. Sci. 29, 1275 (2006).
10. 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).
11. Wittwer, et al. High-resolution genotyping by amplicon melting analysis using LCGreen. Clin. Chem, 49, 853(2003).
12. 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
31000	EvaGreen® Dye, 20X in Water
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_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
41028	Agarose LE, Ultra-Pure Molecular Biology Grade
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.