Revised: December 3, 2014

Product Information

EvaGreen® Dye, 25 mM in DMSO

Catalog Number: 31002

Unit Size: 1 mL

Molecular Information: Proprietary

Color and Form: Orange solution

Spectral Property: $\lambda_{abs}/\lambda_{em}$ = 500/530 nm (DNA bound);
λ_{abs} = 471 nm (without DNA)

Storage and Handling

EvaGreen® dye is very stable. Store at room temperature protected from light. Product is stable for at least 12 months from the date of receipt when stored as recommended.

The 25 mM concentrated solution can also be stored at 4°C or -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 1:1000 in 1X PBS buffer (pH 7.4), the absorbance of the dye solution at 469 nm should be 2 +/- 0.15.

For convenience, the 25 mM concentrated solution my be diluted 100 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 make the dye useful for several applications including qPCR[1,2], melt curve analysis[3], real-time monitoring of thermophilic helicase-dependent amplification (tHDA)[4], routine solution DNA quantification[5,6] and capillary gel electrophoresis[7,8]. 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 nonfluorescent by itself, but becomes highly fluorescent upon binding to dsDNA. EvaGreen dye is nonmutagenic and noncytotoxic by being completely impermeable to cell membranes (Figure 3), unlike SYBR Green I, which enters cell rapidly and is known to be a powerful mutation-enhancer[9].

For detailed information on the dye's use in qPCR, please refer to EvaGreen Dye 20X in water (catalog no. 31000), or one of Biotium's ready-to-use EvaGreen master mixes. If your application is qPCR, we highly recommend that you test at least one of these products before experimenting with EvaGreen dye, 25 mM in DMSO so that you become familiar with the optimal dye concentration as measured by optical density.

Toxicity

Ames test performed by an independent lab, Litron Laboratories (Rochester, NY), showed that EvaGreen dye is nonmutagenic as well as noncytotoxic. 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 el. Mutat. Res. 492, 91(2001)). The toxicity of SYBR Green I may be associated with its ability to enter cells rapidly (Figure 3). For more information, you may download the EvaGreen Safety Report at 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 2.5 uM is classified as nonhazardous for drain disposal under CCR Title 22 regulation (download the EvaGreen Safety Report at www.biotium.com for more information). If required by your local regulations, EvaGreen can be adsorbed onto activated charcoal for disposal. Pour 10 liters of EvaGreen waste solution through ~1 g of activated charcoal. The filtrate may directly go to the drain while the charcoal may be treated as solid chemical waste. Biotium offers activated charcoal decontamination bags (catalog no. 22007) for convenient decontamination of EvaGreen dye and similar dyes.

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 el. Ethidium bromide and SYBR Green I enhance the genotoxicity of UV-irradiation and chemical mutagens in E. coli. Mutat. Res. 492, 91 (2001).

Spectral Characteristics

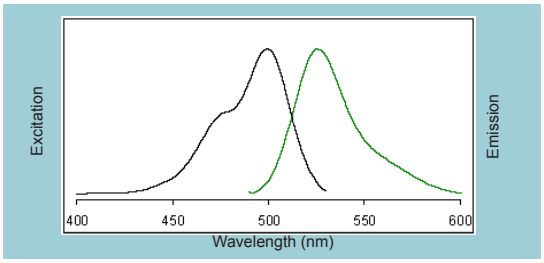

Figure 1. Excitation (left) and emission (right) spectra of EvaGreen dye bound to dsDNA in pH 7.3 PBS buffer.

Stability Comparison of EvaGreen® Dye and SYBR® Green I

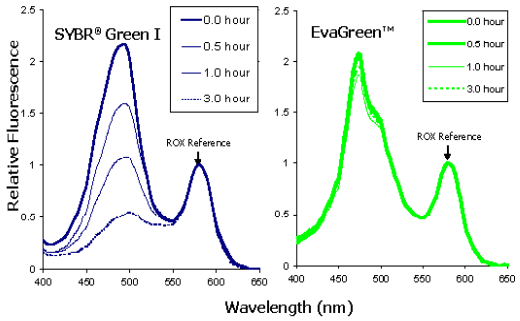

Figure 2. A solution of EvaGreen dye or SYBR Green I each at 1.2 uM in pH 9 Tris buffer was incubated at 99 °C. The absorption spectrum of each solution was followed over a period of 3 hours. ROX was added as a stable reference.

Comparison of Cell Membrane Permeability of EvaGreen® Dye and SYBR® Green I

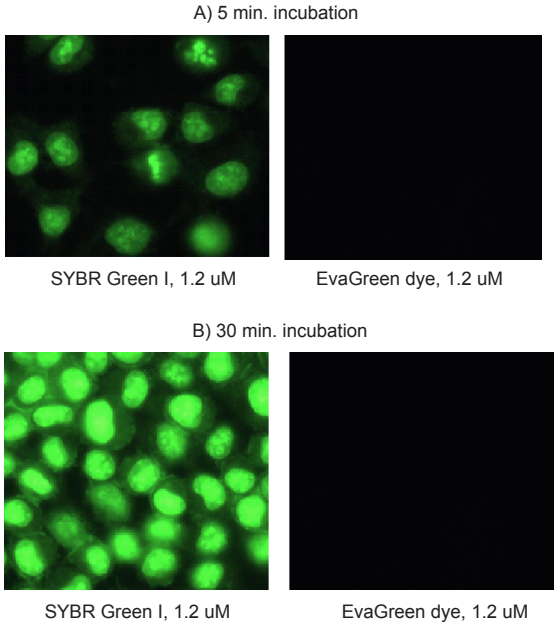

Figure 3. HeLa cells were incubated with SYBR Green I (1.2 uM) or EvaGreen dye (1.2 uM) at 37 °C. Photographs were taken following incubation for 5 min (panel A) and 30 min (panel B). SYBR Green I entered cells rapidly while EvaGreen appeared to be membrane-impermeable.