GENTAUR

DATA SHEET

Product Information

EvaGreen™ Dye, 25 mM in DMSO

Catalog Number: 31002

Packaging 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. We recommend EvaGreen™ dye in DMSO to be stored at 4°C protected from light. The expected shelf-life under the recommended conditions should be at least 12 months from the date of receipt. Under cold storage conditions, dye precipitation may occur, in which case the vial containing the dye may be warmed up to 45°C until the solution becomes clear. For convenience, the 25 mM concentrated solution my be diluted 100 times to a 0.25 mM solution in either di H2O or Tris (10mM, 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 20X qPCR (Cat # 31000) and EvaGreen 2X Basic Mix (Cat #31001). Both of these products are in ready-to-use formats with optimized dye concentrations. If your application is qPCR, we highly recommend that you test at least one of the two 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).

Since these toxicity tests were not performed on human, we still advise that researchers exercise precautions when handling the dye or any other DNA-binding molecules by wearing protective gears. For more information on the Ames test result, you may download a complete report at Biotium website.

Disposal

EvaGreen™ solution may be disposed of using one of the following methods: 1) Add 25~50 mL bleach (regular household bleach) to each gallon (~4L) of the waste solution containing the dye and let the mixture react for at least 8 hours before pouring the solution to a sink; 2) Pour each 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 regular 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 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

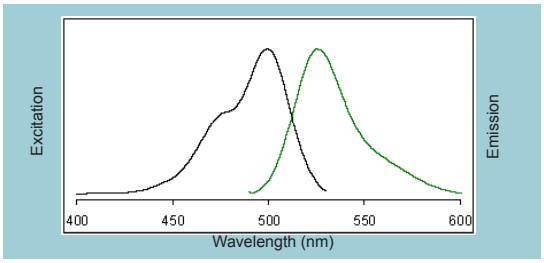


Figure 1. Excitation (left) and emission (right) spectra of EvaGreen™ dye bound to dsDNA in pH 7.3 PBS buffer.

Stability Comparison of EvaGreen™ Dye and SYBR® Green I

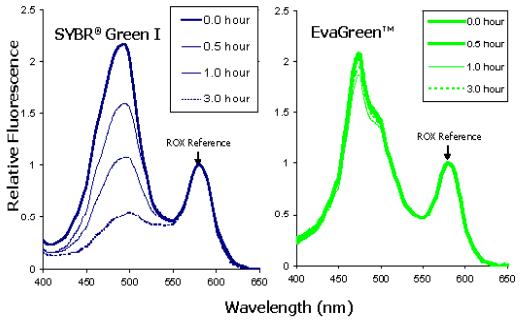
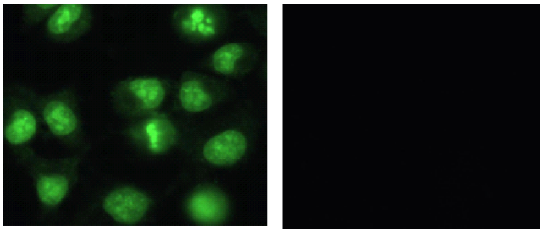


Figure 2. A solution of EvaGreen™ dye or SYBR® Green I each at 1.2 μM in pH 9 Tris buffer was incubated at 99 °C. The absorption spectrum of each solution was followed over a period of 3 hours. ROX was added as a stable reference.

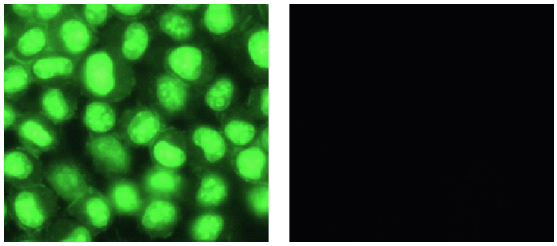
Comparison of Cell Membrane Permeability between EvaGreen™ Dye and SYBR® Green I

A) 5 min. incubation



SYBR Green I, 1.2 μM | EvaGreen dye, 1.2 μM

B) 30 min. incubation



SYBR Green I, 1.2 μM | EvaGreen dye, 1.2 μM

Figure 3. HeLa cells were incubated with SYBR Green I (1.2 μM) or EvaGreen dye (1.2 μM) 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 membrane-impermeable.