GENTAUR

DATA SHEET

Product Information

EvaGreen™ Dye, 20X in water

Catalog Number: 31000

Packaging Size: 5 x 1 mL

Molecular Information: Proprietary*

Color and Form: Light 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™ 20X solution be stored at 4°C or below to prevent mold formation. The expected shelf-life under the recommended condition should be at least 12 months from the date of receipt. When taking the dye solution out of the freezer, vortex the solution for a few seconds in case of dye adsorption on the container wall during storage.

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].

The unique properties of EvaGreen™ dye have made it particularly useful in quantitative real-time PCR (qPCR) application. 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. More significantly, the higher EvaGreen™ concentration permitted for qPCR eliminates "dye redistribution" problems, which can occur with SYBR® Green I during post-PCR DNA melt curve analysis. Dye redistribution problems may make SYBR® Green I unreliable for DNA melt curve analysis (Giglio, et al. Nucleic Acid Res. 31(22), e136(2003); Wittwer, et al. Clin. Chem. 49(6), 853(2003)). On the other hand, EvaGreen™ dye is optimal for both qPCR and melt curve analysis, yielding robust and reproducible results.

EvaGreen™ 20X solution is specifically formulated for qPCR use. PCR reaction can be monitored using your existing optical setting for SYBR® Green I or FAM on any commercial real-time PCR cycler. The qPCR protocol provided below is for PCR using regular non-hot-start Taq. Use of a hot-start Taq may require some adjustment of PCR buffer composition in terms of ionic strength and pH to best take the advantage of EvaGreen™ dye. For example, chemically-modified Taq, such as AmpliTaq Gold, may prefer a downward-adjustment of KCl concentration (to as low as 0.0 mM) and an upward-adjustment of Tris concentration (to as high as 50 mM). In addition, a water soluble solvent such as DMSO or glycerol has traditionally been added to stabilize a master mix. These components plus the pH may need to be optimized depending on the nature of your enzyme. Nevertheless, if you use a regular non-hot-start Taq and follow the protocol provided below, you should expect to see superior performance from EvaGreen™ dye over that from SYBR® Green I. Because the optical settings vary slightly from instrument to instrument and the wavelengths of EvaGreen™ dye are slightly longer than those of SYBR® Green I, Ct value may differ slightly by +1 or –1 when compared with SYBR® Green I side-by-side. However, regardless of which cycler you use, the fluorescence signals with EvaGreen™ dye for both qPCR and melt curve analysis should be significantly stronger than those with SYBR® Green I.

Recommended Protocol for 50 μL-sized Reactions (employing non-hotstart Taq):

1 Set up PCR reaction as follows[1]:
- 5 μL of 10x polymerase buffer without magnesium[2]
- 2.5μL of 50mM $MgCl_2$ [3]
- 5 μL each of 2 mM dNTP
- 2.5 μL of 20X EvaGreen™ [4]
- 1-5 units of Taq DNA polymerase[5]
- 0.1-1 μM each of primers (final concentrations)

Add Di-H_2O to make a final volume of 50 μL.

2 Perform real-time PCR reaction on a thermocycling fluorometer and record the fluorescence signal at the annealing or extension step.

[1] For iCycler users, you do not need to add FAM to your PCR mix since EvaGreen™ has a slight background fluorescence that provides an adequate and stable baseline level fluorescence for well calibration.

When using ABI Sequence Detection Systems, make sure to select NONE for the passive reference under the tab WELL INSPECTOR.

BSA may be required if the reaction is run on a Roche LightCycler. A final BSA concentration of 0.5mg/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. Since EvaGreen™ dye is less sensitive to proteins, you may need to adjust the instrument setting (for background fluorescence) so that the instrument will start.

[2] For chemically-modified Taq, it may be necessary to downward adjust the KCl concentration and upward adjust Tris concentration.

[3] The optimal Mg^{2+} concentration for PCR with EvaGreen™ dye is 2.5 mM.

[4] Before pipetting, warm up the 20X solution to room temperature and thoroughly mix the solution by vortexing. EvaGreen™ is highly stable. However, dye adsorption onto container wall may occur during storage at low temperature over a long period of time. Should it occur, vortexing the vial for a few seconds should alleviate the problem.

[5] For best results, a hot-start enzyme should be used. However, buffer condition may need to be adjusted accordingly to best take the advantage of the dye.

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 physico-chemical 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

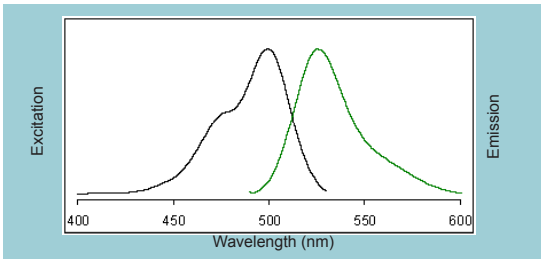

Figure 1. Excitation (left) and emission (right) spectra of EvaGreen™ dye bound to dsDNA in pH 7.3 PBS buffer.

Stability Comparison of EvaGreen™ Dye and SYBR® Green I

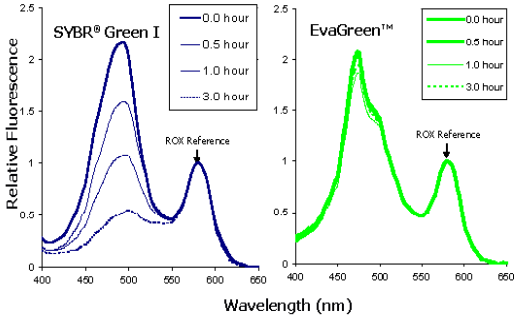

Figure 2. A solution of EvaGreen™ dye or SYBR® Green I each at 1.2 μM in pH 9 Tris buffer was incubated at 99 °C. The absorption spectrum of each solution was followed over a period of 3 hours. ROX was added as a stable reference.

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

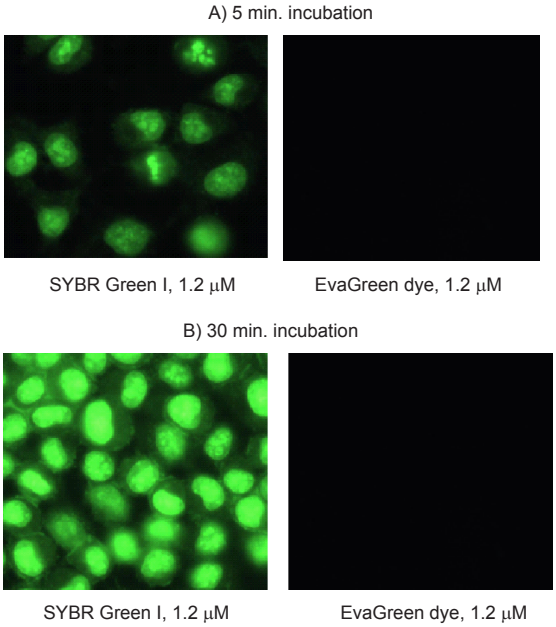

Figure 3. HeLa cells were incubated with SYBR Green I (1.2 μM) or EvaGreen dye (1.2 μM) at 37 °C. Photographs were taken following incubation for 5 min (panel A) and 30 min (panel B). SYBR Green I entered cells rapidly while EvaGreen appeared membrane-impermeable.