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Revised: December 3, 2014

Product Information

EvaGreen® Dye, 2000X in DMSO

Catalog Number: 31019

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 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 1:100 in 1X PBS buffer (pH 7.4), the absorbance of the dye solution at 469 nm should be 2 +/- 0.15.

For convenience, the 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 non-fluorescent by itself, but becomes highly fluorescent upon binding to dsDNA. EvaGreen dye is nonmutagenic and noncytotoxic because it is 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 2000X in DMSO is specifically formulated 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. 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 lower concentration of KCl or no KCl and higher Tris concentration (up to 50 mM). In addition, a water soluble solvent such as DMSO or glycerol are frequently added to stabilize master mixes. These components and pH may need to be optimized depending on the enzyme used.

Using non-hot-start Taq and the protocol provided below, EvaGreen dye shows higher fluorescent signals than SYBR Green I for both qPCR and melt curve analysis. 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.

Protocol

The following protocol is recommended for use with non-hotstart Taq.
Note: The recommended concentration of EvaGreen dye for qPCR is 1X. The protocol below uses a diluted stock solution of 20X EvaGreen dye in water.

1. Set up the 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)
   dH_2O to a final volume of 50 µL.

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

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

Note: 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.

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

[2] For chemically-modified Taq, it may be necessary to reduce the KCl concentration and increase the 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 may adsorption to the vial during storage. Vortex the vial for a few seconds to make sure the dye is fully dissolved.

[5] For best results, a hot-start enzyme should be used. However, buffer formulation may need to be adjusted.

Safety

Ames testing performed by an independent lab, Litron Laboratories (Rochester, NY), showed that EvaGreen dye is nonmutagenic as well as non-cytotoxic. EvaGreen dye appears to be completely cell membrane-impermeable (Figure 3), which may be a key factor responsible for the observed low toxicity.

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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). You can download a complete safety report on EvaGreen dye 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 2X concentration (2.5 uM) is classified as nonhazardous for drain disposal under CCR Title 22 regulation. 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).

Related Products

Cat. No.	Description
31000	EvaGreen® Dye, 20X in water, 5 X 1mL
29050	Cheetah™ HotStart Taq DNA Polymerase, 500 U
29052	ROX reference dye, 25 uM in TE buffer, 5 X 1 mL
31003	Fast EvaGreen® qPCR Master Mix, 200 reactions
31020	Fast Plus EvaGreen® qPCR Master Mix, No Rox, 200 reactions
31014	Fast Plus EvaGreen® qPCR Master Mix, Low Rox, 200 reactions
31015	Fast Plus EvaGreen® qPCR Master Mix, High Rox, 200 reactions
31005	Fast Probe qPCR Master Mix, No Rox, 200 reactions
31016	Fast Probe qPCR Master Mix with Rox, 200 reactions
41003	GelRed™ Nucleic Acid Gel Stain, 10,000X in H_2O, 0.5 mL
41005	GelGreen™ Nucleic Acid Gel Stain, 10,000X in H_2O, 0.5 mL
31030	DNA Gel Extraction Kit, 50 or 250 columns
31039	1 KB DNA Ladder in TE Buffer (100 ng/uL), 30 ug/300 ul
31022	Ready-to-Use 1 KB DNA Ladder, 150 applications (1.5 ml)
31040	100 bp DNA Ladder in TE Buffer (100 ng/uL), 30 ug/300 ul
31032	Ready-to-Use 100 bp DNA Ladder, 150 applications (1.5 ml)
41006	TBE Buffer, 5X, 4L
22007	Activated charcoal decontamination bags, 25 items

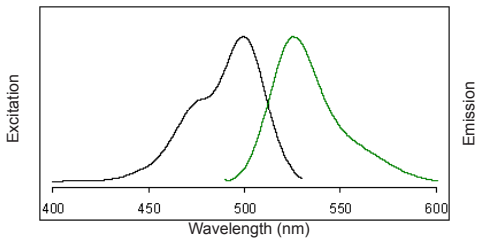

Figure 1. Excitation (left) and emission (right) spectra of EvaGreen™ dye bound to dsDNA in pH 7.3 PBS buffer.

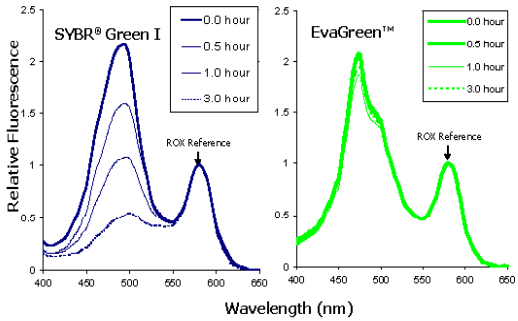

Figure 2. Stability comparison of EvaGreen and SYBR Green dyes. Solutions of EvaGreen dye or SYBR Green I at 1.2 μM in pH 9 Tris buffer were incubated at 99 °C. The absorption spectrum of each solution was measured over a period of 3 hours. ROX was added as a stable reference.

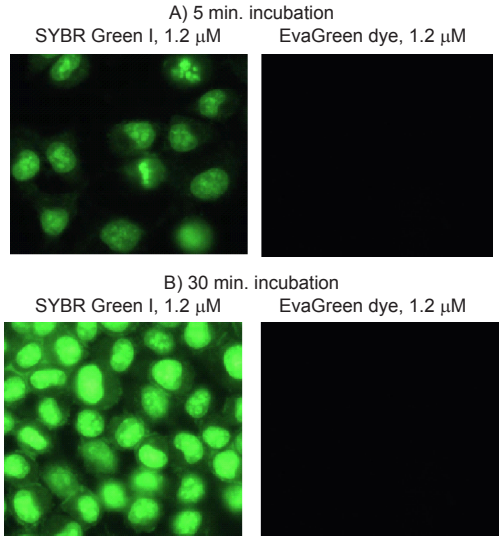

Figure 3. Cell permeability of EvaGreen and SYBR Green dyes. HeLa cells were incubated with SYBR Green I (1.2 μM) or EvaGreen dye (1.2 μM) at 37 °C. Images were taken following incubation for 5 min (panel A) and 30 min (panel B). SYBR Green I entered cells rapidly while EvaGreen was be membrane-impermeable.