EvaGreen® Dye, 20X in Water

Catalog Number: 31000-T, 31000

Unit Size
31000-T: 1 mL
31000: 5 x 1 mL

Concentration: 20X (25 uM) in water

Color and Form: Light orange solution

Spectral Properties
\[ \lambda_{\text{abs}} = 471 \text{ nm (without DNA)} \]
\[ \lambda_{\text{em}} / \lambda_{\text{abs}} = 500/530 \text{ nm (with DNA)} \]

Storage and Handling
Store at 4°C, protected from light. EvaGreen® Dye is extremely stable both thermally and chemically (1), however, because this product is preservative-free, after opening we recommend storing in aliquots at -20°C to avoid contamination.

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® (5), LAMP (6), 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 20X in Water is a convenient concentration 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. An example protocol provided is for qPCR using Biotium’s Cheetah™ HotStart Taq; qPCR conditions may require optimization for specific targets or sample types.

We also offer EvaGreen® Dye in a 200X concentration in DMSO for when higher concentrations are needed, 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 20X 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 Mg²⁺ 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 MgCl₂
   - 0.1-1 uM each of primers
   - 0.2 mM each of dNTPs
   - 0.02-0.1 unit/µL Cheetah™ HotStart Taq DNA Polymerase
   - 1X EvaGreen® Dye
   - Optional ROX Reference Dye (if required by your instrument)
   - dH₂O 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, Liton 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.
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 a list of selected references for various applications.


Related Products

<table>
<thead>
<tr>
<th>Catalog number</th>
<th>Product</th>
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<tbody>
<tr>
<td>31019</td>
<td>EvaGreen® Dye, 2000X in DMSO</td>
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<td>31077</td>
<td>EvaGreen® Plus Dye, 20X in Water</td>
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<td>29050</td>
<td>Cheetah™ HotStart Taq DNA Polymerase, 500 U</td>
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<td>29052</td>
<td>ROX reference dye, 25 uM in TE buffer</td>
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<td>Forget-Me-Not™ EvaGreen® qPCR Master Mix</td>
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<td>31041, 31042</td>
<td>Forget-Me-Not™ EvaGreen® qPCR Master Mix, (2-Color Tracking)</td>
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<td>GelRed® Nucleic Acid Gel Stain, 3X in H₂O</td>
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<td>GelRed® Nucleic Acid Gel Stain, 10.00X in H₂O</td>
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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.

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