Revised: March 11, 2020

Product Information

EvaGreen® Plus Dye, 20X in Water

Catalog Number: 31077-T, 31077

Size
31077-T: 1 mL
31077: 5 x 1 mL

Spectral Properties
Ex = 460 nm (without DNA)
Ex/Em = 487/525 nm (with DNA; see Fig. 1)

Storage and Handling
Store at -20°C, protected from light. EvaGreen® Plus Dye is very stable and may also be stored at 4°C, however, because this product is preservative-free, we recommend storing at -20°C to avoid contamination. Product is stable for at least 12 months from the date of receipt when stored as recommended. No information is available concerning potential hazards of EvaGreen® Plus Dye. Exercise universal laboratory safety precautions during handling, and dispose as hazardous chemical waste.

Product Description
EvaGreen® Plus Dye is an improved alternative to Biotium's original EvaGreen® Dye. EvaGreen® Plus Dye retains many of the essential benefits of original EvaGreen® Dye, but has an improved signal-to-noise ratio that is more advantageous for a variety of DNA detection applications, including qPCR, digital PCR, and LAMP.

Similar to the original EvaGreen® Dye, EvaGreen® Plus Dye is a green fluorescent nucleic acid dye that is essentially nonfluorescent by itself, but becomes highly fluorescent upon binding to dsDNA. However, EvaGreen® Plus Dye improves upon these properties with a lower background fluorescence and an increased brightness upon binding dsDNA. Additionally, it has an excitation maximum at 487 nm (Fig. 1), which is a better match for common qPCR instrument excitation sources; further increasing the dye's signal. As a result, EvaGreen® Plus Dye can give earlier Ct values and better signal discrimination. Other DNA amplification and detection assays should experience comparable improvement to the results generated (Figs. 2, 3).

The unique properties of Biotium's EvaGreen® dyes make them particularly useful in quantitative real-time PCR applications. Relative to other green dyes, such as SYBR® Green I, both EvaGreen® dyes are generally less inhibitory toward PCR and less likely to cause nonspecific amplification. Both EvaGreen® dyes enable direct visualization of the PCR product on a gel using a UV transilluminator or blue light box without the need for another gel stain. Furthermore, EvaGreen® dyes can be used at a much higher dye concentration, resulting in more robust PCR signal than other qPCR dyes.

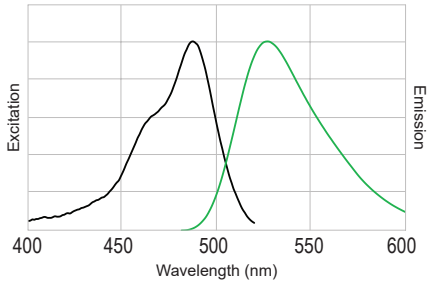

Figure 1. Excitation (left) and emission (right) spectra of EvaGreen® Plus dye bound to dsDNA in 1M Tris buffer, pH 8.5.

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.
- EvaGreen® Plus Dye can be used for high resolution melting (HRM®) analysis. Follow your qPCR system's instructions for data collection and analysis.

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 $MgCl_2$
   0.1-1 uM each of primers
   0.2 mM each of dNTPs
   0.02-0.1 unit/uL Cheetah™ HotStart Taq DNA Polymerase
   1X EvaGreen® Plus Dye
   Optional ROX Reference Dye (see Table 1)

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® Plus 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.

Table 1. Instrument Compatibility

Reference Dye	PCR Instrument
Low ROX (~50 nM)	Applied Biosystems®: 7500, 7500 Fast, ViiA™7, QuantStudio™ instruments Stratagene (Agilent): MX4000P, MX3000P, MX3005P
High ROX (~500 nM)	Applied Biosystems®: 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™, StepOnePlus™
No ROX required	BioRad: iCycler™, MyiQ™, MiQ™ 2, iQ™ 5, CFX-96 Touch™, CFX-384 Touch™ and Connect™, Chromo4™, MiniOpticon™ Qiagen: Rotor-Gene® Q, Rotor-Gene® 3000 & 6000 Eppendorf: Mastercycler® Realplex Illumina: Eco™ RealTime PCR System Cepheid: SmartCyler® Roche: LightCycler® 480, LightCycler® 2.0

Related Products

Catalog number	Product
31019	EvaGreen® Dye, 2000X in DMSO
31000	EvaGreen® Dye, 20X in Water
29050	Cheetah™ HotStart Taq DNA Polymerase
29052	ROX Reference Dye, 25 uM in TE Buffer
29051	EvaEZ™ Fluorometric Polymerase Activity Assay Kit
31045, 31046	Forget-Me-Not™ EvaGreen® qPCR Master Mix
31041, 31042	Forget-Me-Not™ EvaGreen® qPCR Master Mix, (2-Color Tracking)
31043	Forget-Me-Not™ Universal Probe qPCR Master Mix
31028	AccuClear® Ultra High Sensitivity dsDNA Quantitation Kit
31006	AccuBlue® High Sensitivity dsDNA Quantitation Kit
31007	AccuBlue® Broad Range dsDNA Quantitation Kit
31060	AccuBlue® NextGen dsDNA Quantitation Kit
31069	AccuGreen™ Broad Range dsDNA Quantitation Kit (for Qubit®)
31066	AccuGreen™ High Sensitivity dsDNA Quantitation Kit (for Qubit®)
31073	AccuBlue® Broad Range RNA Quantitation Kit
CD201	RNAstorm™ Kit for Isolation of RNA from FFPE Tissue Samples
CD202	DNAstorm™ Kit for Isolation of RNA from FFPE Tissue Samples
41024-4L	Water, Ultrapure Molecular Biology Grade (4L Cubitainer®)
41003	GelRed® Nucleic Acid Gel Stain, 10,000X in Water
41005	GelGreen® Nucleic Acid Gel Stain, 10,000X in Water
41028	Agarose LE, Ultra-Pure Molecular Biology Grade
41029	GelRed® Agarose LE
41030	GelGreen® Agarose LE
E90003	Gel-Bright™ LED Gel Illuminator
31022	Ready-to-Use 1 kb DNA Ladder
31032	Ready-to-Use 100 bp DNA Ladder
41006	TBE Buffer, 5X (4L Cubitainer®)

Please visit our website at www.biotium.com for information on our life science research products, including DNA & RNA quantitation kits, fluorescent CF® dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, environmentally friendly EvaGreen® qPCR master mixes, and other reagents for molecular and cell biology research.

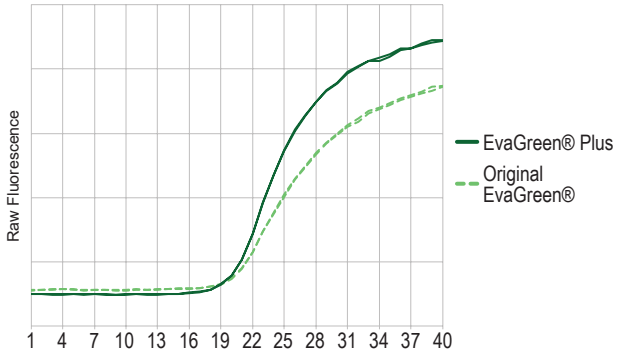

Figure 2. A comparison of the raw fluorescence signals from qPCR reactions performed with EvaGreen® Plus Dye (Solid, Dark Green) and original EvaGreen® Dye (Dashed, Light Green), two replicates each. EvaGreen® Plus Dye gives an earlier Ct, has a lower background, and higher maximum signal intensity.

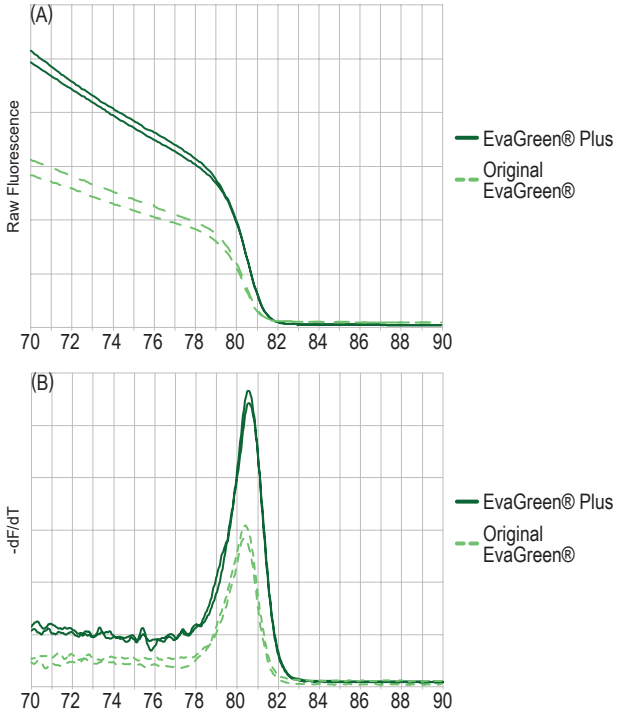

Figure 3. A comparison of EvaGreen® Dyes during melt curve analysis. EvaGreen® Plus Dye (Solid, Dark Green) and original EvaGreen® Dye (Dashed, Light Green), two replicates each. The raw fluorescence melt plot (A) reveals that EvaGreen® Plus Dye is brighter when bound to dsDNA and has a lower post-melt background fluorescence. The derivative melt plot (B) displays no significant shift to melt temperatures or DNA disassociation characteristics.