

Appendix. EvaGreen Dye® Characteristics

The following figures provide additional information on EvaGreen® dye in regard to its spectra, stability and cell membrane permeability.

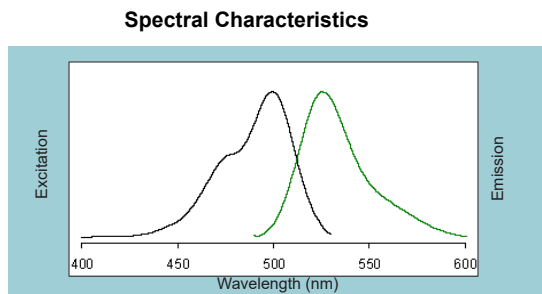


Figure 1. Excitation (left) and emission (right) spectra of EvaGreen® dye bound to dsDNA in pH 7.3 PBS buffer. Also see ref. 1.

Stability Comparison of EvaGreen® Dye and SYBR® Green I

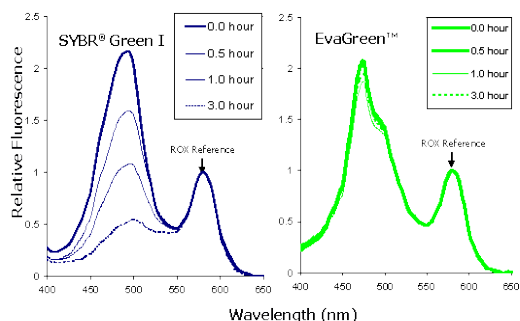


Figure 2. A solution of EvaGreen® dye or SYBR® Green I each at 1.2 μ M in pH 9 Tris buffer was incubated at 99 °C. The absorption spectrum of each solution was followed over a period of 3 hours. ROX was added as a stable reference. SYBR Green I nearly completely disappeared while no decomposition was noticeable for EvaGreen dye.

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

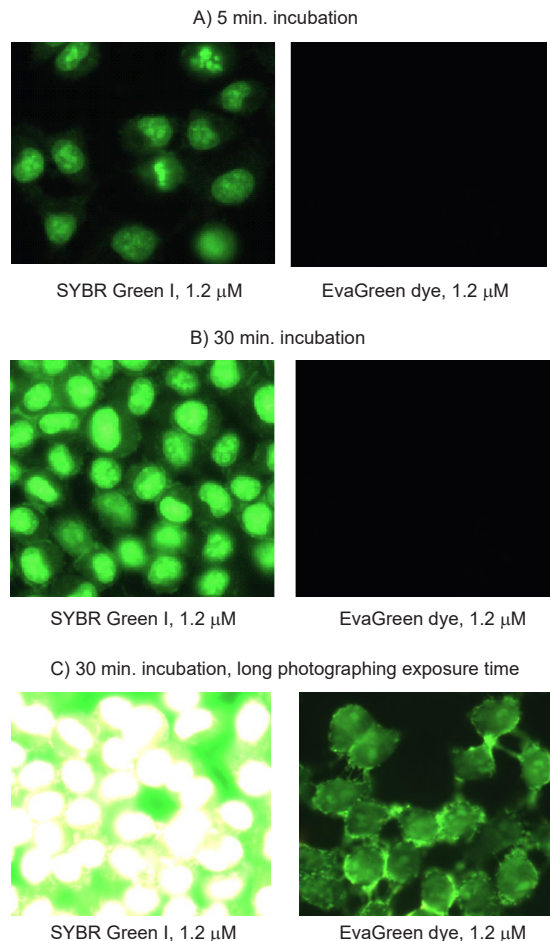


Figure 3. HeLa cells were incubated with SYBR Green I (1.2 μ M) or EvaGreen dye (1.2 μ M) at 37 °C. Photographs were taken following incubation for 5 min (panel A) and 30 min (panels B and C). SYBR Green I entered cells rapidly while EvaGreen appeared membrane-impermeable as evident from the absence of cell nuclear staining (panels A and B). Image taking with long exposure time revealed that EvaGreen dye only associated with cell membranes (panel C). SYBR Green I has been suggested to interfere with the DNA repair mechanism in living cells, a rationale used to explain the observation that the dye is even more environmentally toxic than the widely known mutagen ethidium bromide (Ohta, et al. *Mutation Research*, **492**, 91-97(2001)). In contrast, EvaGreen dye has been confirmed to be nonmutagenic and nontoxic. See EvaGreen safety report at Biotium website.

Biotium products are high-quality reagents and materials intended for research purposes only. EvaGreen® dye and applications are covered under patent US patent nos. 7,803,943 and 7,776,567 and pending international patents. Our products are not available for resale or other commercial uses without a specific agreement from Biotium, Inc. We welcome inquiries about licensing the use of our dyes, trademarks or technologies. Please submit inquiries by e-mail to btinfo@biotium.com. EvaGreen® is a registered trademark of Biotium, Inc.

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