Cyber Green™ *20X Aqueous PCR Solution*

Ordering Information	*Storage Conditions*
Product Number: 17591 (5 mL in H_2O, 20X)	Keep in -20°C. Avoid exposure to light

Biological Applications

Cyber Green™ dye is a green fluorescent nucleic acid dye with features that make the dye useful for several applications including qPCR, melt curve analysis, real-time monitoring of thermophilic helicase-dependent amplification (tHDA), routine solution DNA quantification, and capillary gel electrophoresis. The DNA-bound dye has excitation and emission spectra very close to those of fluorescein (FAM) or SYBR® Green I, making the dye compatible with instruments equipped with the 488 nm argon laser or any visible light excitation with wavelength in the region. Cyber Green™ dye is extremely stable both thermally and hydrolytically, providing convenience during routine handling. The dye is essentially non-fluorescent by itself, but becomes highly fluorescent upon binding to dsDNA. The unique properties of Cyber Green™ dye have made it particularly useful in quantitative real-time PCR (qPCR) application. Compared with the widely used SYBR Green I, Cyber Green™ is generally less inhibitory toward PCR and less likely to cause nonspecific amplification. As a result, Cyber Green™ can be used at a much higher dye concentration than SYBR Green I, resulting in more robust PCR signal. More significantly, the higher Cyber Green™ concentration permitted for qPCR eliminates "dye redistribution" problems, which can occur with SYBR Green I during post-PCR DNA melt curve analysis.

Cyber Green™ 20X in water 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 Cyber Green™. 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 is frequently added to stabilize master mixes. These components and pH may need to be optimized depending on the enzyme used.

General Properties

Ex/Em = 497/521 nm

Sample Protocol

The following protocol is recommended for use with non-hot start Taq.
1. Set up the PCR reaction as follows:

5 µL of 10x polymerase buffer without Mg^{+2}
2.5µL of 50mM $MgCl_2$
5 µL each of 2 mM dNTP
2.5 µL of 20X Cyber Green™
1-5 units of Taq DNA polymerase
0.1-1 µM each of primers (final concentrations)
ddH2O 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.