Cyber Green™ Nucleic Acid Gel Stain *10,000X* (Equivalent to SYBR® Green)

Ordering Information	*Storage Conditions*
Product Number: 17590 (1 mL in DMSO, 10,000X)	Keep in -20°C. Avoid exposure to light

Biological Applications

Cyber Green™ dye is a green fluorogenic nucleic acid dye that can be used for qPCR, melt curve analysis, real-time monitoring of thermophilic helicase-dependent amplification (tHDA), routine solution DNA quantification, and a variety of gel electrophoresis. The DNA-bound Cyber Green™ dye has excitation and emission spectra very close to those of fluorescein (FAM) or SYBR® Green, 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 fluorescent intensity is at least an order-of-magnitude greater than that of ethidium bromide. Also, the fluorescence quantum yield of the DNA/ Cyber Green™ complex is more than 5 times greater than that of DNA/ethidium bromide. It can be used before electrophoresis (prestaining), as well as after electrophoresis (poststaining), and stain DNA separated by capillary electrophoresis. It is also commonly used in molecular biology with no enzymes (eg : Taq enzyme , reverse transcriptase, endonuclease , T4 ligase) inhibition, and does not interfere with Southern blotting techniques.

Spectral Properties

Ex/Em = 497/521 nm when bound to DNA

Handling and Disposal

Cyber Green™ I nucleic acid gel stain is significantly less mutagenic than ethidium bromide. However, we must caution that no data are available on the mutagenicity or toxicity of Cyber Green™ I stain in humans. It should be treated as a potential mutagen and used with appropriate care due to the fact that this reagent binds to nucleic acids. Dispose of the stain in compliance with local regulations.

Staining Protocols

We have found the greatest sensitivity is achieved by post-staining which also eliminates the possibility of dye interference with DNA migration. While the precast protocol is more convenient, some DNA samples may experience migration, it is highly recommend the gel running time does not exceed more than 2 hours. The following protocols are recommended. However some comparisons might be needed to determine which one better meets your needs.

1. **Post-staining Protocol**

1.1 Run gels based on your standard protocol.

1.2 Make 1X Cyber Green™ working solution by diluting the 10,000X stock reagent into PH 7.5 - 8 buffer (e.g., TAE, TBE or TE preferably pH 8.0).

Note: Staining solutions prepared in water are less stable than those prepared in buffer and must be used within 24 hours to ensure maximal staining sensitivity. In addition, staining solutions prepared in buffers with pH below about 7.5 or above 8.0 are less stable and show reduced staining efficacy.

1.3 Place the gel in a suitable polypropylene container. Gently add a sufficient amount of the 1X staining solution to submerge the gel.

Note: Do not use a glass container, as it will adsorb much of the dye in the staining solution.

1.4 Agitate the gel gently at room temperature for ~30 minutes, protecting from the light.

Note: The staining solution may be stored in the dark (preferably refrigerated) for a week and reused up to 2-3 times.

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1.5 Image the stained gel with a 254 nm transilluminator, or a laser-based gel scanner using a long path green filter such as a SYBR® filter or GelStar® filter.

2. Pre-casting protocol

2.1 Prepare agarose gel solution using your standard protocol.

2.2 Dilute the 10,000X Cyber Green™ stock reagent into the gel solution at 1:10,000 just prior to pouring the gel and mix thoroughly.

2.3 Run gels based on your standard protocol.

2.4 Image the stained gel with a 254 nm transilluminator, or a laser-based gel scanner using a long path green filter such as a SYBR® filter or GelStar® filter.

3. DNA-staining before electrophoresis

3.1 Incubate DNA with a 1:10,000 dilution of the dye (in TE, TBE, or TAE) for at least 15 minutes prior to electrophoresis.

3.2 Run gels based on your standard protocol.

3.3 Image the stained gel with a 254 nm transilluminator, or a laser-based gel scanner using a long path green filter such as a SYBR® filter or GelStar® filter.

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