SYTOX® Green Nucleic Acid Stain

Cat. No. PA-3019

Storage upon receipt: -20°C

Protect from light

Ex/Em: 504/523 nm (bound to DNA)

Instructions for Use

SYTOX Green nucleic acid stain is a high-affinity nucleic acid stain that easily penetrates cells with compromised plasma membranes and yet will not cross the membranes of live cells. It is suitable for use with eukaryotic cells and is particularly useful with both gram-positive and gram-negative bacteria, where an exceptionally bright signal is required.[1,2] After brief incubation with SYTOX Green nucleic acid stain, the nucleic acids of dead cells fluoresce bright green when excited with the 488 nm spectral line of the argon-ion laser, or any other 450–490 nm source. These properties, combined with its >500-fold fluorescence enhancement upon nucleic acid binding, make the SYTOX Green stain a simple and quantitative single-step dead-cell indicator for use with fluorescence microscopes, fluorometers, fluorescence microplate readers and flow cytometers.

This dead-cell stain may be used in conjunction with blue and red-fluorescent surface labels for multiparameter analyses. It may also be possible to combine SYTOX Green nucleic acid stain with DAPI for two-color visualization of dead and live cells. SYTOX Green nucleic acid stain is also an excellent DNA counterstain for chromosome labeling and for fixed cells and tissues.

Contents, Storage and Handling

The SYTOX Green dye is supplied as a 5 mM solution in dimethylsulfoxide (DMSO) in a unit size of 250 µl. Upon receipt, this vial should be stored frozen at -20°C, upright and protected from light. Before refreezing, seal the vial tightly.

The DMSO solution may be subjected to many freeze-thaw cycles without reagent degradation. When stored properly, this stock solution is stable for at least one year. Each vial contains enough reagent to stain

>1500 samples when using a 96-well microplate assay format.

Caution: No data are available addressing the mutagenicity or toxicity of this reagent.

Because the reagent binds to nucleic acids, it should be treated as a potential mutagen and used with appropriate care. The DMSO stock solution should be handled with particular caution, as DMSO is known to facilitate the entry of organic molecules into tissues. We strongly recommend using double gloves when handling the DMSO stock solution. As with all nucleic acid stains, solutions containing this reagent should be poured through activated charcoal before disposal. The charcoal must then be incinerated to destroy the dyes.

Spectral Characteristics

Upon binding DNA, the SYTOX Green dye exhibits a fluorescence enhancement of greater than 500-fold. The SYTOX Green/DNA complex has excitation and emission maxima of 504 nm and 523 nm, respectively, and a fluorescence quantum yield of 0.53. Spectral characteristics of the SYTOX Green dye in bacteria or eukaryotic cells may vary.

Experimental Guidelines

The following procedure can be adapted for any cell type.

Note that different concentration ranges for the SYTOX Green dye are suggested depending on the cell type.

Growth medium, cell density, the presence of other cell types and other factors may influence staining. In general, the best results are obtained in buffers that do not contain phosphate.

Residual detergent on glassware may also affect real or apparent staining of many organisms,

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causing brightly stained material to appear in solutions with or without cells present. Be sure to wash glassware in a mild detergent and rinse thoroughly with hot tap water followed by several rinses with deionized, distilled water. Pellet cells by centrifugation and resuspend in buffered salt solution or water. The binding of SYTOX Green stain may be reduced somewhat in solutions containing very high concentrations of monovalent or divalent cations. Adherent cells may be stained in situ on coverslips. Add SYTOX Green stain using the concentrations listed below as a guide. In initial experiments, it may be best to try several dye concentrations over the entire suggested range to determine the concentration that yields optimal staining. Cells stained with SYTOX Green dye can be viewed with a fluorescence microscope equipped with a standard fluorescein filter set. Stained eukaryotic cells will generally have bright green nuclei as well as some low-level cytoplasmic staining. Bacteria generally stain uniformly once the intracellular dye is at equilibrium with the staining solution. Allow 5 minutes or more for staining of bacteria or eukaryotic cells to reach completion.

Concentration and Incubation Conditions

Bacteria (0.5–5 µM) Vortex to mix then incubate for >5 minutes.

Yeast (1–50 µM) Incubate with periodic agitation for >10 minutes.

Other Eukaryotes (10 nM–1 µM) Incubate for >10 minutes.

References

1. J Appl Bacteriol 81, 411 (1996);
2. J Appl Environ Microbiol 63, 2421 (1997).

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