# Product Data Sheet

Product Name: SYTO-9
Cat. No.: GC79856

## Chemical Properties

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|---|---|---|---|
| Cas. No. | | | |
| Formula | | M.Wt | |
| Solubility | DMSO: 100 mg/mL (Need ultrasonic) | Storage | Store at -20°C, sealed storage, away from moisture and light |
| General tips | For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20°C for several months. | | |
| Shipping Condition | Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request. | | |
| Structure | | | |

## Background

SYTO-9 is a cell-permeable fluorescent dye that can be used for nucleic acid staining. The fluorescence intensity of SYTO-9 increases significantly upon binding to RNA and DNA. SYTO-9 can stain RNA and DNA in live and dead eukaryotic cells, as well as in Gram-positive and Gram-negative bacteria. SYTO-9 is applicable for fluorescence microscopy, flow cytometry, fluorophotometry, and bacterial counting in aquatic systems (RNA: Ex/Em = 486/501 nm; DNA: Ex/Em = 485/498 nm) [1] [2].

***Caution: Product has not been fully validated for medical applications. For research use only.***
***Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com***
***Address: 10292 Central Ave. #205, Montclair, CA, USA***

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In Vitro, Operating Instructions (The following is a recommended experimental protocol for guidance only and should be adjusted according to specific requirements) 1. Working Solution Preparation 1.1 Diluent: Phosphate-buffered saline (PBS) or HBSS is optional. 1.2 Working concentration: 0.5-5 μM for single staining; 33.4 μM for co-staining with propidium iodide (PI). 1.3 Notes: The concentration of the working solution can be adjusted as needed, and it must be prepared and used immediately. 2. Staining Procedures 2.1 Suspended bacterial samples[1][2][3]: 2.1.1 Sample preparation: Adjust the bacterial suspension to a concentration of approximately 105-108 CFU/mL in the selected diluent; if removal of culture medium or fixative (if applicable) is required, centrifuge at 4000-8000 × g for 2-10 min before resuspension; centrifugation is not required for direct staining of live or formaldehyde-fixed aquatic bacteria. 2.1.2 Incubation conditions: Use a working concentration of 0.5-33.4 μM; incubate at room temperature in the dark for 5-45 min; for pure bacterial cultures, perform staining in amber tubes to avoid light exposure. For PI co-staining, use a combined system of 33.4 μM SYTO-9 and 0.4 mM PI, and incubate at room temperature in the dark for 15 min. 2.2 Adherent bacterial biofilms[4]: 2.2.1 Sample preparation: Use biofilms grown on silicon wafers, coverslips or glass-bottom dishes; trypsin digestion is not required. 2.2.2 Washing before staining: Wash the samples 3 times with PBS. 2.2.3 Incubation conditions: Direct co-staining with SYTO-9-PI: Use the prepared co-staining working solution and incubate at room temperature for 15 min. Sequential staining: First incubate with SYTO-9 working solution at room temperature for 15 min, then directly add PI and image immediately; or after staining with DAPI and PI, directly add SYTO-9 and image immediately. 2.2.4 Washing after staining: Wash the samples 3 times with PBS to remove excess dye. 3. Control Setup 3.1 Bacterial samples without dye. 3.2 Blank control containing only peptone water. 3.3 Peptone water solution containing 0.5 μM SYTO-9. 3.4 Peptone water solution containing 1.0 μM SYTO-9. 3.5 Untreated Staphylococcus aureus biofilm control. 3.6 Staphylococcus aureus biofilm samples treated with 70% EtOH. 4. Detection and Analysis 5.1 Instrument types: Flow cytometer, fluorescence microscope, etc. 4.2 Excitation/emission wavelengths: RNA: Ex/Em = 486/501 nm; DNA: Ex/Em = 485/498 nm. 4.3 Result analysis: After SYTO-9 binds to bacterial nucleic acids, the fluorescence intensity increases significantly, and the intensity varies with bacterial species, cell concentration, and the state of live/fixed bacteria. 4.3.1 Suspended bacteria: Staining duration (5-45 min) has minimal effect on fluorescence intensity, but gram-negative bacteria may show low signals at 5 min; the fluorescence intensity of live bacteria may be higher than that of fixed bacteria; double staining with PI allows differentiation between live and dead bacteria via intensity ratio or spectral analysis. 4.3.2 Biofilms: SYTO-9 can stain intracellular nucleic acids (intact cells appear solid green) or substances around cells (damaged cells appear hollow green); co-staining with PI shows overlapping green and magenta fluorescence, and SYTO 9 signals often distribute around PI signals; fluorescence intensity has no

direct correlation with cell size.

References:

[1]. McGoverin C, et al. Species Dependence of SYTO 9 Staining of Bacteria. Frontiers in microbiology. 2020;11:545419.
[2]. Lebaron P, et al. Comparison of blue nucleic acid dyes for flow cytometric enumeration of bacteria in aquatic systems. Applied and environmental microbiology. 1998 May;64(5):1725-30.
[3]. Ou F, et al. Rapid and cost-effective evaluation of bacterial viability using fluorescence spectroscopy. Analytical and bioanalytical chemistry. 2019 Jun;411(16):3653-3663.
[4]. Luo J, et al. Correlative Imaging and super resolution microscopy studies reveal complexities in determining live-dead state of bacteria. Biofilm. 2025 Dec;10:100302.