DiTO™-3 [equivalent to TOTO®-3] *5 mM DMSO Solution*

Catalog number: 17576
Unit size: 0.2 ml

Component	Storage	Amount
DiTO™-3 [equivalent to TOTO®-3] *5 mM DMSO Solution*	Freeze (< -15 °C), Minimize light exposure	1 vial (0.2 ml)

OVERVIEW

DiTO™-3 is chemically equivalent to TOTO®-3 (TOTO® is the trademark of Invitrogen). DiTO™-3 is a carbocyanine dimer with far-red fluorescence similar to Cy® 5 dyes. It is cell-impermeant and easily distinguished from fluorescein and rhodamine as a nuclear counterstain and dead cell indicator. It is non-fluorescent in the absence of nucleic acids, and generates a very bright fluorescence signal upon binding to DNA. DiTO™-3 gives strong and selective nuclear staining in cultured cells and in paraffin sections. Simultaneous labeling with cell-permeable Nuclear Green™ LCS1 dye and cell-impermeant DiTO®-3 can be used to assess cell viability. DiTO™-3 and Nuclear Green™ both have much greater extinction coefficients than that of DNA-bound propidium iodide.

KEY PARAMETERS

Fluorescence microscope

Excitation	Cy5 filter set
Emission	Cy5 filter set
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Cy5 filter set

PREPARATION OF WORKING SOLUTION

DiTO™-3 working solution
Make DiTO™-3 working solution in Hanks with 20 mM Hepes buffer (HH buffer) or buffer of your choice at your desired concentration.

Note In initial experiments, it may be best to try several dye concentrations to determine the optimal concentration that yields the desired result. High dye concentration tends to cause nonspecific staining of other cellular structures.

SAMPLE EXPERIMENTAL PROTOCOL

Caution: The following protocol can be adapted for most cell types. Growth medium, cell density, the presence of other cell types and factors may influence staining. Residual detergent on glassware may also affect staining of many organisms, and cause brightly stained material to appear in solutions with or without cells present.

1. Grow and treat cells as desired.
2. Remove the cell culture medium and fix cells.
3. Add DiTO™-3 working solution (1 to 10 µM) into the cells (either suspension or adherent cells), and stain the cells for 15 to 60 minutes.
4. Remove the dye working solution and add HH buffer or buffer of your choice.
5. Analyze the cellular staining with a fluorescence microscope using Cy5 filter.

EXAMPLE DATA ANALYSIS AND FIGURES

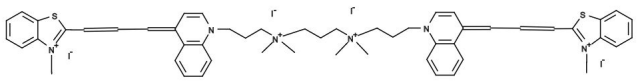

Figure 1. Chemical structure for DiTO™-3 [equivalent to TOTO®-3] *5 mM DMSO Solution*

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.