AAT Bioquest®

PRODUCT INFORMATION SHEET

TWO-PRO™ 3 [equivalent to TO-PRO®-3] *5 mM DMSO Solution* *CAS 157199-63-8*

Catalog number: 17572
Unit size: 0.2 ml

Component	Storage	Amount
TWO-PRO™ 3 [equivalent to TO-PRO®-3] *5 mM DMSO Solution* *CAS 157199-63-8*	Freeze (< -15 °C), Minimize light exposure	1 vial (0.2 mL)

OVERVIEW

TWO-PRO™-3 is chemically equivalent to TO-PRO®-3 (TO-PRO® is the trademark of Invitrogen). TWO-PRO™-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. TWO-PRO™-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 TWO-PRO™-3 can be used to assess cell viability. TWO-PRO™-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

TWO-PRO™-3 working solution
Make TWO-PRO™-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.
3. Add TWO-PRO™-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

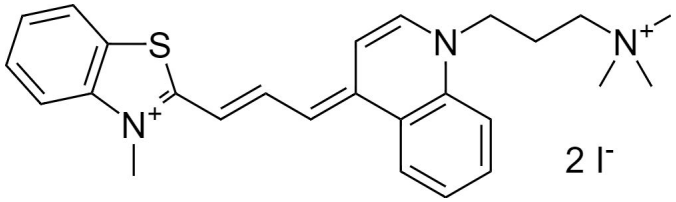

Figure 1. Chemical structure for TWO-PRO™ 3 [equivalent to TO-PRO®-3] *5 mM DMSO Solution* *CAS 157199-63-8*

DISCLAIMER

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