

**LysoBrite™ Red DND-99**

 Catalog number: 22647  
 Unit size: 500 Tests

Component	Storage	Amount (Cat No. 22647)
LysoBrite™ Red DND-99	Freeze (< -15 °C), Minimize light exposure	500 Tests

**OVERVIEW**

LysoBrite Red DND-99 is chemically same to the LysoTracker® Red DND-99 used for labeling and tracking acidic organelles in live cells (LysoTracker® is the trademark of ThermoFisher). It has good selectivity for acidic organelles. The LysoBrite™ probes consist of a fluorophore linked to a weak base that is only partially protonated at neutral pH, allowing them to freely permeate cell membranes to label live cells.

**Chemical and Physical Properties of LysoBrite™ Dyes**

Cat#	Dye	Unit	Mol. Wt.	Ex/Em (nm)
22641	LysoBrite™ NIR	500 tests	~700	636/651 nm
22642	LysoBrite™ Blue	500 tests	~350	434/480 nm
22643	LysoBrite™ Green	500 tests	~450	501/510 nm
22644	LysoBrite™ Orange	500 tests	~700	543/565 nm
22645	LysoBrite™ Red	500 tests	~700	576/596 nm
22646	LysoBrite™ Deep Red	500 tests	~800	597/619 nm
22647	LysoBrite™ Red DND-99	500 tests	~400	573/595 nm
22648	LysoBrite™ Green DND-26	500 tests	~400	501/509 nm

**AT A GLANCE**
**Assay Protocol with LysoBrite™ Red DND-99**

1. Prepare cells.
2. Add dye working solution.
3. Incubate at 37 °C for 30 minutes.
4. Wash the cells.
5. Analyze under a fluorescence microscope.

**Storage and Handling Conditions**

The LysoBrite™ Red DND-99 stock solution provided is 500X in DMSO. It should be stable for at least 6 months if stored at -20°C and protected from light. Avoid freeze/thaw cycles.

**KEY PARAMETERS**
**Fluorescence microscope**

Emission	TRITC filter set
Excitation	TRITC filter set
Recommended plate	Black wall/clear bottom

**Flow cytometer**

Emission	585/40 nm filter
Excitation	532/561 nm laser

**PREPARATION OF WORKING SOLUTION**
**Prepare LysoBrite™ Red DND-99 Working Solution**

1. Warm LysoBrite™ Red DND-99 dye to room temperature.
2. Dilute 20 µL of 500X LysoBrite™ Red DND-99 with 10 mL of Hanks and 20 mM HEPES buffer (HBSS) or a buffer of your choice.

**Note:** 20 µL of LysoBrite™ Red DND-99 dye is enough for one 96-well plate. Aliquot and store unused LysoBrite™ dye stock solutions at < -15 °C. Protect it from light and avoid repeated freeze-thaw cycles.

**Note:** The optimal concentration of the fluorescent lysosome indicator varies depending on the specific application. The staining conditions may be modified according to the particular cell type and the permeability of the cells or tissues to the probe.

**SAMPLE EXPERIMENTAL PROTOCOL**

This protocol only provides a guideline and should be modified according to your specific needs.

**Protocol for Preparing and Staining Adherent Cells**

1. Grow cells in a 96-well black wall/clear bottom plate (100 µL/well/96-well plate) or on coverslips inside a petri dish filled with the appropriate culture medium.
2. When cells reach the desired confluence, add an equal volume of the dye-working solution (from Preparation of Working Solution Step 2).
3. Incubate the cells in a 37 °C, 5% CO<sub>2</sub> incubator for 30 minutes.
4. Wash the cells twice with pre-warmed (37 °C) Hanks and 20 mM HEPES buffer (HBSS) or buffer of your choice. Then fill the cell wells with HBSS or growth medium.
5. Observe the cells using a fluorescence microscope fitted with the desired filter set.

**Note:** It is recommended to increase either the labeling

It is recommended to increase either the labeling concentration or the incubation time to allow the dye to accumulate if the cells do not appear to be sufficiently stained.

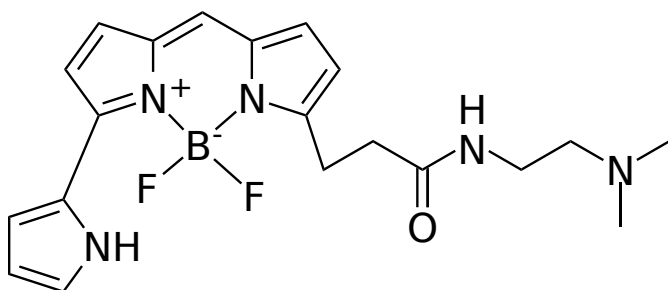
### Protocol for Preparing and Staining Suspension Cells

1. Add an equal volume of the dye-working solution (from Preparation of Working Solution Step 2).
2. Incubate the cells in a 37 °C, 5% CO<sub>2</sub> incubator for 30 minutes.
3. Wash the cells twice with pre-warmed (37 °C) Hanks and 20 mM HEPES buffer (HBSS) or buffer of your choice. Then fill the cell wells with HBSS or growth medium.
4. Observe the cells using a fluorescence microscope fitted with the desired filter set.

**Note:** It is recommended to increase either the labeling concentration or the incubation time to allow the dye to accumulate if the cells do not appear to be sufficiently stained.

**Note:** Suspension cells may be attached to coverslips treated with BD Cell-Tak® (BD Biosciences) and stained as adherent cells (see Protocol for Preparing and Staining Adherent Cells).

### EXAMPLE DATA ANALYSIS AND FIGURES



**Figure 1.** Chemical structure for LysoBrite™ Red DND-99.

### 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](mailto:info@aatbio.com) if you have any questions.