LysoBrite™ Green DND-26

Catalog number: 22648
Unit size: 500 Tests

Component	Storage	Amount (Cat No. 22648)
LysoBrite™ Green DND-26	Freeze (< -15 °C), Minimize light exposure	500 Tests

OVERVIEW

LysoBrite™ Green DND-26 is chemically the same as the LysoTracker® Green DND-26 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.

AT A GLANCE

Assay Protocol with LysoBrite™ Green DND-26

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™ Green DND-26 stock solution provided is 500X in DMSO. It should be stable for at least 6 months if stored at -20°C. Protect from light, and avoid freeze/thaw cycles.

KEY PARAMETERS

Fluorescence microscope

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

Fluorescence microplate reader

Cutoff	515
Emission	525
Excitation	490
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Bottom read mode

Flow cytometer

Emission	530/30 nm filter
Excitation	488 nm laser
Instrument specification(s)	FITC channel

PREPARATION OF WORKING SOLUTION

Prepare LysoBrite™ Green DND-26 working solution

1. Warm LysoBrite™ Green DND-26 dye to room temperature.
2. Prepare dye working solution by diluting 20 µL of 500X LysoBrite™ Green DND-26 with 10 mL of Hanks and 20 mM HEPES buffer (HBSS) or buffer of your choice.

Note: 20 µL of LysoBrite™ Green DND-26 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% CO2 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 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% CO2 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

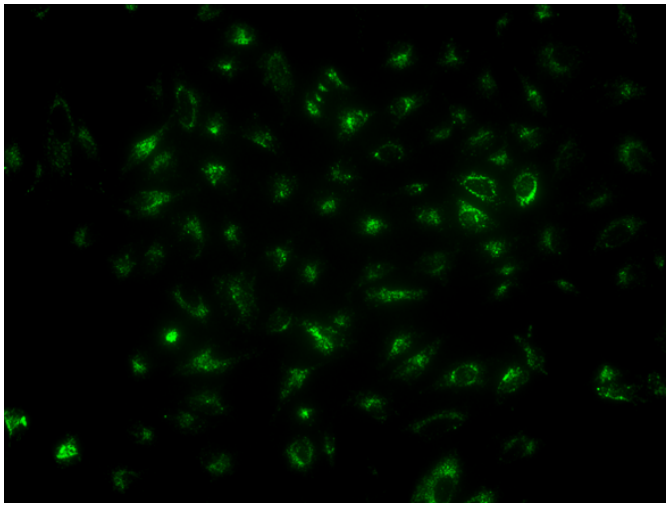

Figure 1. Images of HeLa cells stained with LysoBrite™ Green DND-26 (Cat No. 22648) in a costar black wall/clear bottom 96-well plate. The cells were imaged using a fluorescence microscope with a FITC filter set.

DISCLAIMER

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