# LysoBrite™ Red

Catalog number: 22645
Unit size: 500 Tests

| Component | Storage | Amount (Cat No. 22645) |
|---|---|---|
| LysoBrite™ Red | Freeze (< -15 °C), Minimize light exposure | 500 Tests |

## OVERVIEW

Lysosomes are cellular organelles which contain acid hydrolase enzymes to break up waste materials and cellular debris. Lysosomes digest excess or worn-out organelles, food particles, and engulfed viruses or bacteria. The membrane around a lysosome allows the digestive enzymes to work at pH 4.5. The interior of the lysosomes is acidic (pH 4.5-4.8) compared to the slightly alkaline cytosol (pH 7.2). The lysosome maintains this pH differential by pumping protons from the cytosol across the membrane via proton pumps and chloride ion channels. LysoBrite™ Red selectively accumulates in lysosomes probably via the lysosome pH gradient. The lysotropic indicator is a hydrophobic compound that easily permeates intact live cells, and trapped in lysosomes after it gets into cells. Its fluorescence is significantly enhanced upon entering lysosomes. This key feature significantly reduces its staining background and makes it useful for a variety of studies, including cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis and cytotoxicity. It is suitable for proliferating and non-proliferating cells, and can be used for both suspension and adherent cells. LysoBrite™ dyes significantly outperform the equivalent LysoTracker ™dyes (from Invitrogen). LysoBrite™ dyes can stay in live cells for more than a week with very minimal cell toxicity while the LysoTracker dyes can only be used for a few hours. LysoBrite™ dyes can survive a few generations of cell division. In addition, LysoBrite™ dyes are much more photostable than the LysoTracker dyes.

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

| Cat# | Dye | Unit | Mol. Wt. | Ex (nm) | Em (nm) |
|---|---|---|---|---|---|
| 22641 | LysoBrite™ NIR | 500 tests | ~700 | 636 nm | 650 nm |
| 22642 | LysoBrite™ Blue | 500 tests | ~350 | 433 nm | 480 nm |
| 22643 | LysoBrite™ Green | 500 tests | ~450 | 501 nm | 509 nm |
| 22644 | LysoBrite™ Orange | 500 tests | ~700 | 542 nm | 556 nm |
| 22645 | LysoBrite™ Red | 500 tests | ~700 | 575 nm | 597 nm |
| 22646 | LysoBrite™ Deep Red | 500 tests | ~800 | 596 nm | 619 nm |
| 22647 | LysoBrite™ Red DND-99 | 500 tests | ~400 | 573 nm | 592 nm |
| 22648 | LysoBrite™ Green DND-26 | 500 tests | ~400 | 504 nm | 511 nm |

## AT A GLANCE

**Assay Protocol with LysoBrite™ Red**

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 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 |

**Fluorescence microplate reader**

| | |
|---|---|
| Cutoff | 570 |
| Emission | 590 |
| Excitation | 540 |
| Recommended plate | Black wall/clear bottom |
| Instrument specification(s) | Bottom read mode |

**Flow cytometer**

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

## PREPARATION OF WORKING SOLUTION

**Prepare LysoBrite™ Red Working Solution**

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

   **Note:** 20 µL of LysoBrite™ Red 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

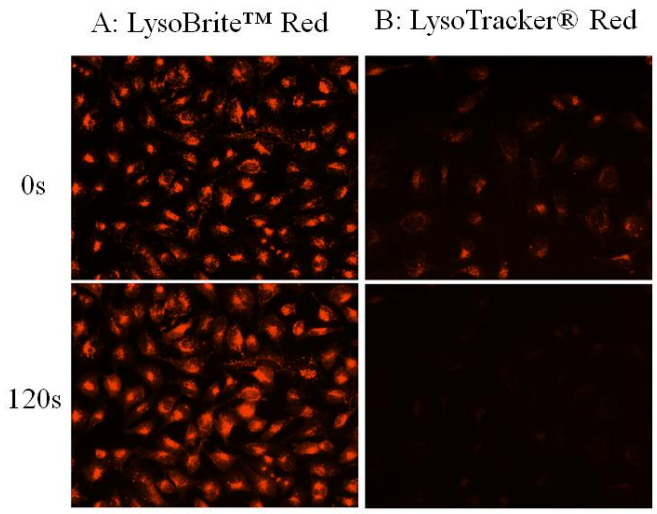


**Figure 1.** Images of HeLa cells stained with A: LysoBrite ™ Red, B: LysoTracker® Red DND-99 (from Invitrogen) in a Costar black wall/clear bottom 96-well plate. Samples were continuously illuminated for 120 seconds, and the signals were compared before and after the exposure by using an Olympus fluorescence microscope.

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