PRODUCT INFORMATION

LysoBrite™ Deep Red

Item No. 25153

Ex./Em. Max: 596/619 nm
Supplied as: A solution in DMSO
Storage: -20°C
Stability: ≥1 year

Information represents the product specifications. Batch specific analytical results are provided on each certificate of analysis.

Description

LysoBrite™ reagents are fluorogenic probes that can be used to label lysosomes within live cells. They are hydrophobic and easily cross live cell membranes to accumulate in lysosomes likely *via* the lysosomal pH gradient. LysoBrite™ fluorescence is enhanced in the acidic environment within the lysosome, and this fluorescence can be measured using fluorescence microscopy, microplate fluorometry, or flow cytometry. LysoBrite™ Deep Red exhibits excitation/emission maxima of 596/619 nm, respectively. It has been used as a lysosome marker to study the localization of hypochlorous acid (HOCl) in live cells.[1]

Assay Protocol

1. **Prepare lysosome-staining solution**
   a. Warm the LysoBrite™ Deep Red solution to room temperature.
   b. Dilute 20 µL of 500X LysoBrite™ Deep Red into 10 mL of Hanks balanced salt solution (HBSS) or buffer of your choice.

Note 1: 20 µL of 500X LysoBrite™ Deep Red yields a volume of lysosome-staining solution sufficient for one 96-well plate. Aliquot and store unused LysoBrite™ Deep Red stock solution at <-15°C. LysoBrite™ Deep Red is light sensitive. Light exposure and repeated freeze-thaw cycles should be avoided

Note 2: The optimal working concentration is application specific. Staining conditions may be modified according to cell type and/or permeability.

2. **Prepare and stain the cells**
   a. Adherent cells:
      i. Grow cells in a 96-well black wall/clear bottom plate (100 µL/well) or on coverslips inside a petri dish filled with appropriate culture medium.
      ii. When cells reach the desired confluency, add 100 µL of the lysosome-staining solution prepared in step 1B.
      iii. Incubate cells at 37°C, 5% CO_2 for 30 minutes.
      iv. Wash cells twice with pre-warmed (37°C) HBSS or buffer of your choice, then fill wells with buffer or growth medium.
      v. Observe cells using fluorescence technique of choice.
   b. Suspension cells*:
      i. Add an equal volume of lysosome-staining solution prepared in step 1B to cells.
      ii. Incubate cells at 37°C, 5% CO_2 for 30 minutes.
      iii. Wash cells twice with pre-warmed (37°C) HBSS or buffer of your choice, then fill wells with buffer or growth medium.
      iv. Observe cells using fluorescence technique of choice.

**Note 3: Suspension cells may be attached to coverslips that have been treated with BD Cell-Tak® (BD Biosciences) and stained as adherent cells.*

Note 4: If cells are not sufficiently stained it is recommended to increase either the labeling concentration or the incubation time to increase cellular dye accumulation.

Reference

1. Shen, S.-L., Ning, J.-Y., Zhang, X.-F., *et al.* Through-bond energy transfer-based ratiometric fluorescent probe for the imaging of HOCl in living cells. *Sens. Actuators B Chem.* **244**, 907-913 (2017).

WARNING
THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

SAFETY DATA
This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

WARRANTY AND LIMITATION OF REMEDY
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