PRODUCT INFORMATION



LysoBrite™ Blue

Item No. 25152

Ex./Em. Max: 353/442 nm Supplied as: A solution in DMSO

-20°C Storage: Stability: ≥2 years

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™ Blue exhibits excitation/emission maxima of 353/442 nm, respectively. It has been used to monitor endosomal escape of doxorubicin theranostic nanoparticles in HeLa cells.¹

Assay Protocol

1. Prepare lysosome-staining solution

- a. Warm the LysoBrite[™] Blue solution to room temperature.
- b. Dilute 20 µl of 500X LysoBrite™ Blue into 10 ml of Hanks balanced salt solution (HBSS) or buffer of your choice.

Note 1: 20 µl of 500X LysoBrite™ Blue yields a volume of lysosome-staining solution sufficient for one 96-well plate. Aliquot and store unused LysoBrite™ Blue stock solution at <15°C. LysoBrite™ Blue 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:

- 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.
- 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% CO₂ 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.
- Observe cells using fluorescence technique of choice.

b. Suspension cells*:

- Add an equal volume of lysosome-staining solution prepared in step 1b to cells.
- Incubate cells at 37°C, 5% CO₂ for 30 minutes.
- 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 cover-slips that have been treated with BD Cell-Tak $^{ exttt{B}}$ (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. Lin, Y., Yang, Y., Yan, J. et al. Redox/ATP switchable theranostic nanoparticles for real-time fluorescence monitoring of doxorubicin delivery J. Mater. Chem. B 6(14), 2089-2103 (2018).

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

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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