

PRODUCT INFORMATION



LysoBrite™ Orange

Item No. 25156

Ex./Em. Max: 542/556 nm
Supplied as: A solution in DMSO
Storage: -20°C
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™ Orange exhibits excitation/emission maxima of 542/556 nm, respectively.

Assay Protocol

1. Prepare lysosome-staining solution

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

Note 1: 20 µl of 500X LysoBrite™ Orange yields a volume of lysosome-staining solution sufficient for one 96-well plate. Aliquot and store unused LysoBrite™ Orange stock solution at less than -15°C. LysoBrite™ Orange 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% 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.
- 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% CO₂ 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.

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.

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