CytoFix™ Red Lysosomal Stain

Catalog number: 23210
Unit size: 500 Tests

Component	Storage	Amount
CytoFix™ LysoRed	Freeze (< -15 °C), Minimize light exposure	1 vial (100 µL)

OVERVIEW

Lysosomes are cellular organelles which contain acid hydrolase enzymes to break up waste and cellular debris through a process known as autophagy. AAT Bioquest offers CytoFix™ Red lysosomal stain for selectively staining lysosomes. CytoFix™ Red lysosomal stain is well retained in lysosomes even after fixation. The dye permeates intact live cells and gets trapped in lysosomes. The fluorescence in lysosomes generated by this dye is well retained at least for 1 week, making it an excellent lysosomal tracking dye. The key features of this stain are its high staining efficiency, long retention after fixation with minimal hands on time. CytoFix™ Red lysosomal stain can be used with GFP expressed cells or with other organelles stains for multicolor analysis. It can be used for both suspension and adherent cells and readily adapted for a wide variety of fluorescence platforms.

AT A GLANCE

1. Prepare cells in growth medium
2. Remove the growth medium
3. Incubate cells with CytoFix™ LysoRed working solution for 20-30 minutes at 37 °C
4. Remove CytoFix™ LysoRed working solution
5. Analyze under fluorescence microscope with Cy3/TRITC filter set

KEY PARAMETERS

Fluorescence microscope

Excitation	Cy3/TRITC filter set
Emission	Cy3/TRITC filter set
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Cy3/TRITC filter set

CELL PREPARATION

For guidelines on cell sample preparation, please visit https://www.aatbio.com/resources/guides/cell-sample-preparation.html

PREPARATION OF WORKING SOLUTION

CytoFix™ LysoRed working solution
Add 20 µL of stock solution into 10 mL Hanks and 20 mM Hepes buffer (HHBS) or buffer of your choice, and mix well.

Note 20 µL stock solution is enough for one 96-well plate assay. The staining conditions may be modified according to the particular cell type and the permeability of the cells or tissues to the probe.

Note Unused CytoFix™ LysoRed stock solution can be aliquoted and stored at ≤ -20 °C with smaller aliquots. Protect from light and avoid repeated freeze-thaw cycles.

SAMPLE EXPERIMENTAL PROTOCOL

1. Prepare cells in growth medium.
2. Remove cell culture medium and wash twice (Optional).
3. Add 100 µL/well (96-well plate) or 50 µL/well (384-well plate) of CytoFix™ LysoRed working solution in the cell plate.

   Note The optimal concentration of the cell membrane probe varies depending on the specific application.
4. Incubate the cells at 37 °C for 20-30 minutes, protected from light.
5. Remove working solution in each well. Wash cells with HHBS or buffer of your choice once (Optional).
6. **Optional:** Fix cells with 4% formaldehyde for 20 minutes at room temperature. Wash cells twice to get rid of any fixation solution.
7. Observe the fluorescence signal in cells using fluorescence microscope with a Cy3/TRITC filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

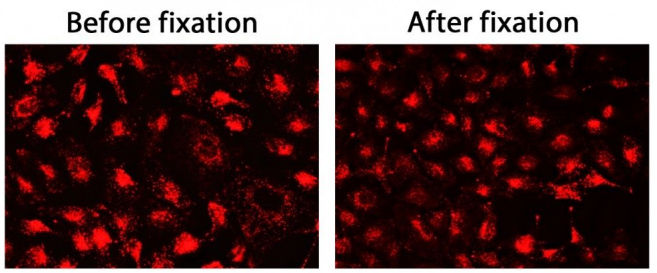

Figure 1. The fluorescence images of HeLa cells stained with CytoFix™ LysoRed in a 96-well black-wall clear-bottom plate. Image was acquired before (Left) and after (Right) fixation with 4% formaldehyde solution for 20 minutes at RT. The cells were imaged using fluorescence microscope with a Cy3/TRITC filter.

DISCLAIMER

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