Actin Lightning™ LC Red *Cell-Permeable*

Catalog number: 22705
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 22705)
Actin Lightning™ LC Red *Cell-Permeable*	Freeze (< -15 °C), Minimize light exposure	100 Tests

OVERVIEW

Actin Lightning™ LC Red is a membrane-permeable fluorescent probe developed for staining filamentous actin (F-actin) in live cells. The dye penetrates intact cell membranes and binds selectively to actin filaments, enabling visualization of cytoskeletal architecture in living cells without the need for fixation during staining. This property allows researchers to monitor actin organization and cellular morphology under physiological conditions using fluorescence microscopy. Unlike other commercially available live-cell actin probes that require efflux pump inhibitors to maintain intracellular signal, Actin Lightning™ LC Red does not require additives such as probenecid or verapamil. The probe can also be used in workflows where cells are subsequently fixed, as the staining signal is retained after fixation. These characteristics support flexible imaging workflows for studying actin dynamics, cytoskeletal rearrangement, and cell morphology in live-cell multiplex experiments.

AT A GLANCE

1. Prepare cells with test compounds at a density of 5 × 10^5 to 1 × 10^6 cells/mL.
2. Prepare and add Actin Lightning™ LC Red working solution to cells.
3. Incubate at 37°C for 30 to 60 minutes.
4. Read fluorescence intensity with Cy5 filter set.

KEY PARAMETERS

Fluorescence microscope

Emission	Cy5 filter set
Excitation	Cy5 filter set
Recommended plate	Black wall/clear bottom

CELL PREPARATION

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

PREPARATION OF STOCK SOLUTIONS

Actin Lightning™ LC Red stock solution (100X):

Prepare stock solution by adding 100 µL of DMSO into the Actin Lightning™ LC Red vial.

Note: *Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles*

PREPARATION OF WORKING SOLUTION

Actin Lightning™ LC Red working solution (1X):

Prepare working solution by adding 10 µL of Actin Lightning™ LC Red stock solution into 1 mL of HHBS (Not provided, AAT Cat# 20011) or buffer of your choice.

Note: Protect the working solution from light by covering it with foil or placing it in the dark. For best results, this solution should be used within a few hours of its preparation.

Note: 1 mL of working solution is enough for 10 tests. The concentration should be optimized based on the staining and cell conditions.

Note: Actin Lightning™ LC Red does not require anion-transporter inhibitors such as probenecid but if cells contain a high number of organic anion-transporters, probenecid (1-2 mM) may be added to the dye working solution (final in-well concentration should be 0.5-1 mM) to reduce leakage of the dye. A variety of ReadiUse™ Probenecid products, including water-soluble, sodium salt, and stabilized solutions, can be purchased from AAT Bioquest.

SAMPLE EXPERIMENTAL PROTOCOL

This protocol only provides a guideline, and should be modified according to your specific needs.

1. Prepare cells in growth medium overnight.
2. Remove the cell culture medium and wash cells with HHBS or buffer of your choice.
3. Add 100 µL of Actin Lightning™ LC Red working solution.
4. Incubate cells in a cell incubator at 37 °C for 30 to 60 minutes.

   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.
5. Remove the dye working solution and wash cells with HHBS or buffer of your choice to remove any excess probes.
6. Image cells with fluorescence microscope using Cy5 filter set.
7. **Optional:** Fix the cells with 4% for later imaging.

EXAMPLE DATA ANALYSIS AND FIGURES

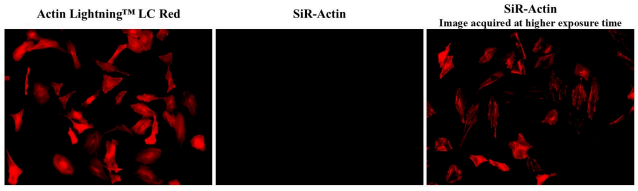

Figure 1. Fluorescence images of live HeLa cells stained with Actin Lightning™ LC Red or SiR-Actin using fluorescence microscope with a Cy5 filter set. The image shown on far left and center was acquired at same exposure time (Exposure time = 1/3 sec) with Cy5 filter. The image on the right used much higher exposure time (Exposure time = 2 sec) with Cy5 filter. SiR-Actin does show very faint staining when image was acquired at higher exposure time.

DISCLAIMER

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