SunRed™ Acetate

Catalog number: 11405
Unit size: 25 mg

Component	Storage	Amount
SunRed™ Acetate	Freeze (<-15 °C), Minimize light exposure	25 mg

OVERVIEW

Esterase-catalyzed hydrolysis of Sun Red acetate (SRA) yields the Sun Red fluorophore that can be excited with the 633 nm laser with emission of ~660 nm. The fluorescence of Sun Red can be readily detected using the Cy5 filter set that is commonly equipped with most of the commercial fluorescence instruments. Although Sun Red is readily excited at 633 nm with red emission of ~660 nm, SRA has very minimal absorption at 633 nm without red emission, making SRA one of the most sensitive NIR esterase substrates. SRA provides a second color for cell viability assay while the popular fluorescein color can used for another cellular functional assay. SRA is a non-fluorescent hydrophobic compound that can pass through the cell membrane whereupon intracellular esterases hydrolyze the acetate group producing the highly fluorescent product Sun Red. The Sun Red molecule accumulates in cells that possess intact membranes so the deep red fluorescence can be used as a marker of cell viability. Cells that do not possess an intact cell membrane or an active metabolism may not accumulate the fluorescent product and therefore do not exhibit deep red fluorescence. SRA may be used in combination with a green vital stain such as a FITC or Alexa Fluor 488-labeled antibody.

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds
2. Remove the medium
3. Add SunRed™ Acetate working solution (100 µL/well/96-well plate or 25 µL/well/384-well plate)
4. Incubate at 37°C, 5% CO_2 incubator for 1 hour
5. Wash and replace the working solution with HHBS
6. Read fluorescence intensity at Ex/Em = 620/660 nm (cut off 640 nm)

Important The following is the recommended protocol. It only provides a guideline, should be modified according to the specific needs.

KEY PARAMETERS

Instrument:	Fluorescence microscope
Excitation:	620 nm
Emission:	660 nm
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	Cy5 filterset

Instrument:	Fluorescence microplate reader
Excitation:	620 nm
Emission:	660 nm
Cutoff:	640 nm
Recommended plate:	Solid black

PREPARATION OF STOCK SOLUTIONS

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.

1. SunRed™ acetate stock solution (2 to 10 mM)
Prepare a 2 to 10 mM stock solution of SunRed™ acetate in DMSO. The stock solution should be used promptly.

PREPARATION OF WORKING SOLUTION

SunRed™ acetate working solution:
Prepare SunRed™ acetate working solution at 5 to 10 µM in 1X Hank's salt solution with 20 mM Hepes buffer (HHBS) or buffer of your choice before the experiment.

PREPARATION OF CELL SAMPLES

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

SAMPLE EXPERIMENTAL PROTOCOL

1. Remove the medium from the cells.
2. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of SunRed™ acetate working solution.
3. Incubate the SunRed™ acetate working solution plate at room temperature or 37°C for 1 hour, protected from light. The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment. For non-adherent cells, it is recommended to centrifuge cell plates at 800 rpm for 2 minutes with brake off after incubation.
4. Monitor the fluorescence intensity at Ex/Em = 620/660 nm (cut off 640 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

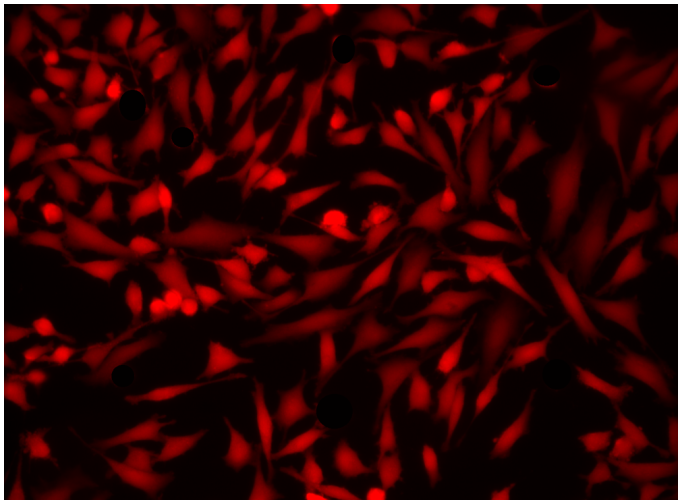

Figure 1.

Fluorescence images of HeLa cells stained with SunRed™ Acetate in a Costar black wall/clear bottom 96-well plate.

DISCLAIMER