HCS LipidTOX™ Neutral Lipid Stains

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
HCS LipidTOX™ Green neutral lipid stain	125 µL	Solution in DMSO (1000X for steatosis assay; 200X for adipogenesis assay)	• <–20°C • Desiccate • Protect from light • Avoid freeze/thaw cycles	Product is shipped at room temperature. When stored as directed, reagent is stable for at least 6 months.
HCS LipidTOX™ Red neutral lipid stain	125 µL	Solution in DMSO (1000X for steatosis assay; 200X for adipogenesis assay	• <–20°C • Desiccate • Protect from light • Avoid freeze/thaw cycles	Product is shipped at room temperature. When stored as directed, reagent is stable for at least 6 months.
HCS LipidTOX™ Deep Red neutral lipid stain	125 µL	Solution in DMSO (1000X for steatosis assay; 200X for adipogenesis assay	• <–20°C • Desiccate • Protect from light • Avoid freeze/thaw cycles	Product is shipped at room temperature. When stored as directed, reagent is stable for at least 6 months.
Number of assays: For the steatosis assay, sufficient reagent is supplied for approximately 1200 assays/10 plates, based on assay volumes of 100 µL per well in 96-well plates. For the adipogenesis assay, sufficient reagent is supplied for approximately 240 assays based on assay volumes of 100 µL.				
Approximate fluorescence excitation/emission maxima: 495/505 nm for LipidTOX™ Green neutral lipid stain; 577/609 nm for LipidTOX™ Red neutral lipid stain; and 637/655 nm for LipidTOX™ Deep Red neutral lipid stain.				

Introduction

The intracellular accumulation of neutral lipids, steatosis, is often triggered by drugs that affect the metabolism of fatty acids and/or neutral lipids. Invitrogen's HCS LipidTOX™ neutral lipid stains were developed for image-based high-content screening (HCS) assays to characterize the potentially toxic side effects of compounds on lipid metabolism in mammalian cell lines. The LipidTOX™ neutral lipid stains have an extremely high affinity for neutral lipid droplets. These reagents are added after fixation of cells and do not require wash steps after addition of the probe. The key advantages of this series of neutral lipid stains over conventional stains such as Nile Red are:

- high specificity to neutral lipid
- ready-to-use formulations for easy assay preparation
- convenience—no wash step required
- mix-and-match multiplexing flexibility

The LipidTOX™ neutral lipid stains are designed for fixed–end point workflows in which formaldehyde-fixed cells in microplates are processed, imaged, and analyzed. The HCS LipidTOX™ neutral lipid stains used for the analysis of steatosis can be easily detected with fluorescence microscopes or HCS readers equipped with standard filter sets (Figure 1). Cellular labeling can be quantified with stand-alone image analysis software or the built-in image analysis software of most HCS readers. In addition, these probes have been developed to be compatible with the HCS LipidTOX™ phospholipidosis detection reagents (H34350 and H34351)

HCS LipidTOX™ neutral lipid stains can also be used to monitor the formation and differentiation of adipocytes (Figure 2), a process called adipogenesis. Adipogenesis is of acute interest to the biomedical and drug discovery community as it plays an important role in diseases such as obesity, diabetes, and atherosclerosis.

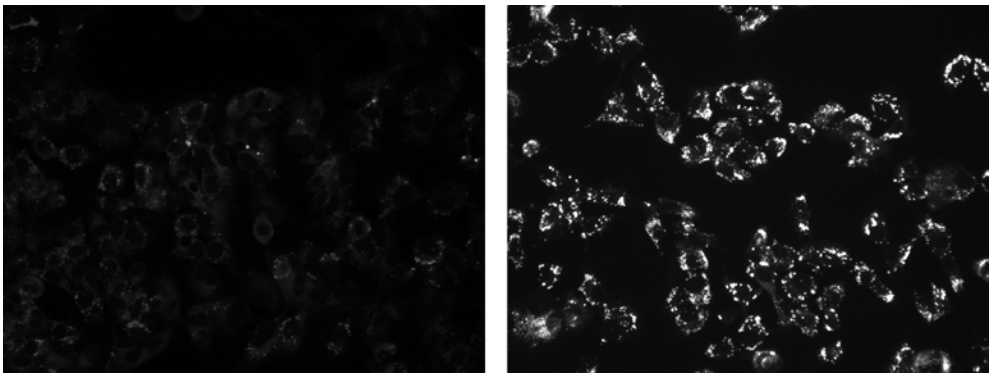

Figure 1. Steatosis detection. Live human hepatocellular carcinoma cells (Hep G2) were untreated (Panel A) or treated with 10 µM cyclosporin A (Panel B) for 48 hours. Cells were formaldehyde-fixed and neutral lipid accumulation was detected with LipidTOX™ Green neutral lipid stain according to the protocol in this manual. Similar data were obtained with both LipidTOX™ Red and Deep Red neutral lipid stains.

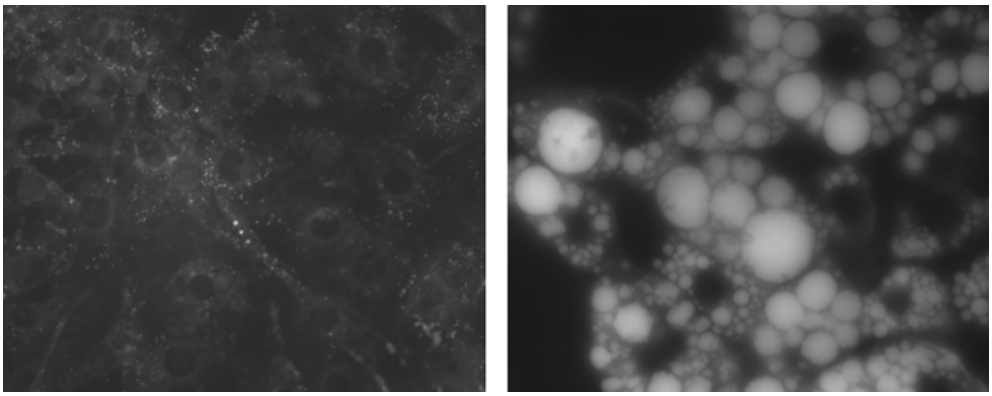

Figure 2. Neutral lipid staining of adipocytes. 3T3-L1 fibroblasts were untreated (Panel A) or differentiated into adipocytes (Panel B). Cells were formaldehyde-fixed and labeled with LipidTOX™ Deep Red neutral lipid stain according to the protocol in this manual. Similar data were obtained with both LipidTOX™ Green and Red neutral lipid stains.

Before You Begin

Allow the vial to warm to room temperature before opening.

Caution

Please handle all the HCS LipidTOX™ neutral lipid stains using good laboratory practice and dispose of them in accordance with local regulations.

Preparing the Cell Line of Interest

For steatosis assays, seed cells in microplates the day before test compound addition. Any cell number and plate coating requirements should be optimized for the chosen cell model. The protocols below were developed using Hep G2 cells, for which it is highly recommended that cells are seeded on collagen I–coated plates at a density of ≤5000 cells/well for 96-well plates the day before treatment. For adipogenesis assays, cells may be prepared on coverslips or microplates for image-based analysis or in suspension for flow cytometric analysis. Refer to *Staining Protocol for Adipogenesis* (in this manual) for a general protocol to label adipocytes.

Experimental Protocols

Staining Protocol for Steatosis

The protocol below describes neutral lipid staining for cells grown in 96-well plates. If immunofluorescence staining is to be performed, avoid agents such as Triton® and Tween® detergents, instead use either saponin or digitonin for permeabilization.

Note: The following steps should be performed after test compound and control incubation is complete. The control compound that we recommend for inducing steatosis is cyclosporin A.

Formaldehyde Fixation

1.1 Prepare a 3.0–4.0% solution of formaldehyde in buffer. A volume of 100 µL/well is required

1.2 Remove the incubation medium, add 100 µL of formaldehyde fixative solution to each well, and incubate for 10–30 minutes at room temperature.

1.3 Remove the fixative solution and rinse the formaldehyde-fixed cells gently with buffer 2–3 times to remove residual formaldehyde before labeling with the neutral lipid staining solution.

Preparing the Labeling Solution and Staining the Cells

2.1 Dilute the 1000X LipidTOX™ neutral lipid stain 1:1000 in buffer to make a 1X solution. A volume of 100 µL/well is required.

2.2 Remove buffer from the cells (after washes from step 1.3) and add 100 µL of 1X LipidTOX™ neutral lipid stain to each well.

2.3 RECOMMENDED: Seal the plates with plate-sealing film.

2.4 Incubate the plates at room temperature for at least 30 minutes before imaging and image the plate without washing. Proceed to *Image Acquisition and Analysis*.

Staining Protocol for Adipogenesis

While the protocol below describes neutral lipid staining for cells differentiated into adipocytes on coverslips, it is also possible to use the stains in other formats such as microplate-based assays and flow cytometric analysis. If immunofluorescence staining is to be performed, avoid agents such as Triton® and Tween® detergents, instead use either saponin or digitonin for permeabilization.

Formaldehyde Fixation

3.1 Prepare a 3.0–4.0% solution of formaldehyde in buffer.

3.2 Remove the incubation medium, add enough formaldehyde fixative solution to cover cells, and incubate for 10–30 minutes at room temperature.

3.3 Remove the fixative solution and rinse the formaldehyde-fixed cells gently with buffer 2–3 times to remove residual formaldehyde; proceed to step 4.1.

Preparing the Labeling Solution and Staining the Cells

4.1 Dilute the LipidTOX™ neutral lipid stain 1:200 in buffer. Prepare a volume sufficient to completely cover cells.

Note: The 1:200 dilution is a recommended starting point. It may be necessary to optimize the dilution for your system.

4.2 Remove the buffer from the cells (after washes from step 3.3)

4.3 Add LipidTOX™ neutral lipid stain and incubate the cells at room temperature for at least 30 minutes before imaging. Proceed to *Image Acquisition and Analysis*.

Image Acquisition and Analysis

We recommend imaging the cells within a week after processing.

LipidTOX™ Green neutral lipid stain can be imaged with filter sets appropriate for Alexa Fluor® 488 dye or fluorescein. LipidTOX™ Red neutral lipid stain is best imaged with filter sets appropriate for Alex Fluor® 594 dye or Texas Red® dye. LipidTOX™ Deep Red neutral lipid stain can imaged with filter sets appropriate for Alexa Fluor® 647 dye or Cy®5 dye.

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat #	Product Name	Unit Size
H34475	HCS LipidTOX™ Green neutral lipid stain *solution in DMSO* *for cellular imaging*	each
H34476	HCS LipidTOX™ Red neutral lipid stain *solution in DMSO* *for cellular imaging*	each
H34477	HCS LipidTOX™ Deep Red neutral lipid stain *solution in DMSO* *for cellular imaging*	each
H34350	HCS LipidTOX™ Green phospholipidosis detection reagent *1000X aqueous solution* *for cellular imaging* *10-plate size*	each
H34351	HCS LipidTOX™ Red phospholipidosis detection reagent *1000X aqueous solution* *for cellular imaging* *10-plate size*	each
H34157	HCS LipidTOX™ Phospholipidosis and Steatosis Detection Kit *for high content screening* *for cellular imaging* *2-plate size*	1 kit
H34158	HCS LipidTOX™ Phospholipidosis and Steatosis Detection Kit *for high content screening* *for cellular imaging* *10-plate size*	1 kit
H32711	HCS CellMask™ Red cytoplasmic/nuclear stain *5 mM solution in DMSO* *for high content screening* *for cellular imaging*	125 µL
H34558	HCS CellMask™ Blue cytoplasmic/nuclear stain *for high content screening* *for cellular imaging*	1 set
H34560	HCS CellMask™ Deep Red cytoplasmic/nuclear stain *for high content screening* *for cellular imaging*	1 set

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