HCS LipidTOX™ Phospholipidosis Detection Reagents

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
HCS LipidTOX™ Green phospholipidosis detection reagent	125 µL	1000X aqueous stock	• <–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 phospholipidosis detection reagent	125 µL	1000X aqueous stock	• <–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: sufficient reagent is supplied for approximately 1200 assays/10 plates based on assay volumes of 100 µL per well in 96-well plates.				
Approximate fluorescence excitation/emission maxima: 495/525 nm for LipidTOX™ Green phospholipidosis detection reagent and 595/615 nm for LipidTOX™ Red phospholipidosis detection reagent.				

Introduction

The intracellular accumulation of phospholipids, phospholipidosis, is often triggered by cationic amphiphilic drugs and can be detected in cells incubated in the presence of phospholipids conjugated to fluorescent dyes.[1] Invitrogen's HCS LipidTOX™ phospholipidosis detection reagents 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 key advantages of this series of phospholipidosis detection reagents over conventional stains such as NBD-PE include:

- ready-to-use aqueous formulations for easy assay preparation
- mix-and-match multiplexing flexibility
- narrow emission profiles

These reagents are designed for fixed–end point workflows in which formaldehyde-fixed cells in microplates are processed, imaged, and analyzed. HCS LipidTOX™ phospholipidosis detection reagents 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. LipidTOX™ phospholipidosis detection reagents do not affect the normal growth of cells, and the staining observed after incubation in live cells is maintained after formaldehyde fixation, compatible with workflows for larger-scale screening.

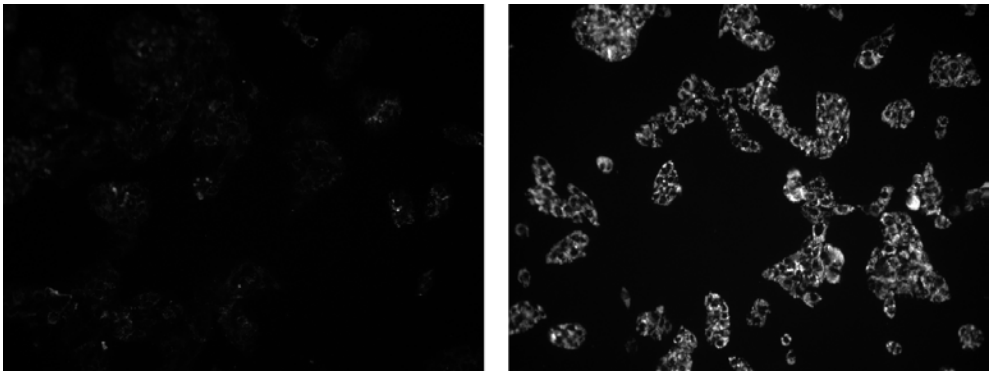

Figure 1. Phospholipidosis detection. Live human hepatocellular carcinoma cells (Hep G2) were untreated (Panel A) or treated with 10 µM amiodarone (Panel B) for 48 hours. Phospholipid accumulation was detected with LipidTOX™ Green phospholipidosis detection reagent according to the protocol in this manual. Similar data were obtained with LipidTOX™ Red phospholipidosis detection reagent.

Before You Begin

Allow the vial to warm to room temperature before opening.

Caution

Please handle all the HCS LipidTOX™ phospholipidosis detection regents using good laboratory practice and dispose of them in accordance with local regulations.

Aggregates in Thawed LipidTOX™ Stain Solutions

After thawing LipidTOX™ phospholipidosis detection reagents some minute aggregates might be observed in the solution. They will usually disappear if the vial is incubated at 37°C for 5 minutes. The aggregates do not affect the performance of the assay. Any aggregates that remain after the stain has been diluted in media are removed by 0.2 µm filtration before the labeling solution is added to the cells, as recommended in the protocol.

Preparing the Cell Line of Interest

Seed cells in microplates the day before test compound and probe addition. Any cell number and plate coating 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.

Experimental Protocols

Volumes given in the protocols below are 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.

Preparing the Labeling Solution and Staining the Cells

1.1 Dilute the 1000X LipidTOX™ phospholipidosis detection reagent 1:500 in normal growth medium to make a 2X solution. A volume of 50 µL/well (after 0.2 µm filtration) is required.

1.2 Filter the 2X labeling solution in normal growth medium with a 0.2 µm filter approved for cell culture use. A volume of 50 µL/well is required.

1.3 Remove medium from the wells of the microplate.

1.4 To all wells, add 50 µL/well 2X LipidTOX™ phospholipidosis detection reagent (prepared in step 1.1 and 1.2).

1.5 To the appropriate wells containing LipidTOX™ phospholipidosis detection reagent, add 50 µL/well of the growth medium containing 2X test compound. The concentration of stain and of test compound in these wells should now be 1X. We recommend amiodarone and propranolol as control compounds for inducing phospholipidosis.

1.6 Incubate the cells under normal culture conditions for a time period sufficient for assessing the effects of the test compound.

Note: a minimum incubation time of 24 hours is recommended, typically 48 to 72 hours incubation is used, depending on the test compound.

Formaldehyde Fixation

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

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

2.3 Remove the fixative solution and rinse the formaldehyde-fixed cells gently with buffer 2–3 times to remove residual formaldehyde. If labeling only with LipidTOX™ phospholipidosis detection reagent, the plate can be sealed with plate-sealing film at this stage before analysis. See *Image Acquisition and Analysis*.

2.4 At this point, steatosis can be analyzed using LipidTOX™ Green, Red, or Deep Red neutral lipid stains (Cat. no. H34475, H34476, or H34477).

Image Acquisition and Analysis

We recommend imaging the cells as soon as possible after processing. If the plate cannot be imaged immediately, processed plates should be kept refrigerated and imaged within one week.

LipidTOX™ Green phospholipidosis detection reagent can be imaged with filter sets appropriate for Alexa Fluor® 488 dye or fluorescein. LipidTOX™ Red phospholipidosis detection reagent is best imaged with filter sets appropriate for Alexa Fluor® 594 dye or Texas Red® dye.

References

1. Toxicology Methods, 1(2): 89-105, 1991.

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

Cat #	Product Name	Unit Size
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
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
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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