# HCS LipidTOX™ Phospholipidosis and Steatosis Detection Kits

**Table 1.** Contents and storage information.

| HCS LipidTOX™ Phospholipidosis and Steatosis Detection Kit | | | | |
|---|---|---|---|---|
| **Material** | **Amount** | **Concentration** | **Storage** | **Stability** |
| LipidTOX™ Red phospholipidosis detection reagent | 25 µL or 125 µL | 1000X aqueous stock | • ≤–20°C<br>• Desiccate<br>• Protect from light<br>• Avoid freeze/thaw cycles | Kit is shipped at room temperature. When stored as directed, kit is stable for at least 6 months.. |
| LipidTOX™ Green neutral lipid stain | 25 µL or 125 µL | 1000X DMSO stock | | |
| Hoechst 33342 | 25 µL or 125 µL | 1000X aqueous stock | | |
| propranolol | 35 µg or 106 µg | NA | | |
| cyclosporin A | 144 µg or 432 µg | NA | | |
| DMSO | 50 µL or 250 µL | NA | | |
| **Number of assays:** sufficient reagents are supplied for 240 assays/2 plates (H34157) or 1,200 assays/10 plates (H34158) based on assay volumes of 100 µL per well | | | | |
| **Approximate fluorescence excitation/emission maxima: 595/615** nm for LipidTOX™ Red phospholipidosis detection reagent; **495/505** nm for LipidTOX™ Green neutral lipid stain; and **352/461** for Hoechst 33342 | | | | |

## Introduction

Invitrogen's HCS LipidTOX™ Phospholipidosis and Steatosis Detection Kits offer the reagents for performing image-based high-content screening (HCS) assays to characterize the potentially toxic side effects of compounds on lipid metabolism in mammalian cell lines. These kits are designed for fixed–end point workflows in which formaldehyde-fixed cells in microplates are processed, imaged, and analyzed. The fluorescent stains used for the analysis of phospholipidosis and steatosis can be easily detected with fluorescence microscopes or HCS readers equipped with standard filter sets. Cellular labeling can be quantified with stand-alone image analysis software or the built-in image analysis software of most HCS readers. A workflow diagram is shown in Figure 1.

The intracellular accumulation of phospholipids, phospholipidosis, is often triggered by cationic amphiphilic drugs and can be detected when cells are incubated in the presence of phospholipids conjugated to fluorescent dyes.[1] The intracellular accumulation of neutral lipids, steatosis, is often triggered by drugs that affect the metabolism of fatty acids and/or neutral lipids. The HCS LipidTOX™ Phospholipidosis and Steatosis Detection Kit incorporates two dyes: the LipidTOX™ Red phospholipidosis detection reagent and the LipidTOX™ Green

neutral lipid stain, which has an extremely high affinity for neutral lipid droplets. Also included is Hoechst 33342 for nuclear labeling and two positive control compounds: propranolol and cyclosporin A. Propranolol induces accumulation of phospholipid, and cyclosporin A induces formation of prominent neutral lipid droplets. Cells are first incubated with LipidTOX™ Red phospholipidosis detection reagent (which does not affect the normal growth of cells) in the presence of the test compound. Cells are then fixed and stained with LipidTOX™ Green neutral lipid stain (Figure 2). Hoechst 33342 can be included in the fixation step if nuclear staining is desired.

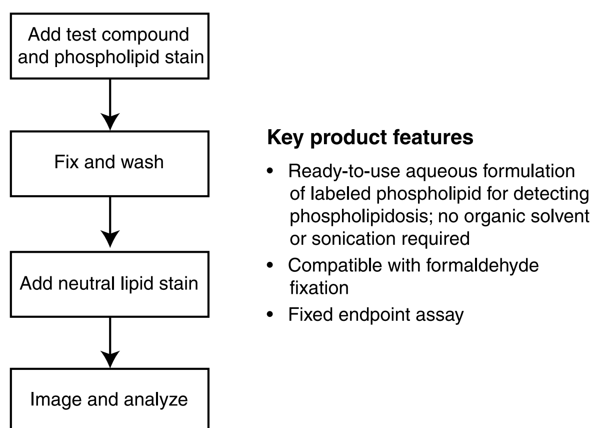


**Figure 1.** Generalized workflow for phospholipid and neutral lipid staining using reagents in the HCS LipidTOX™ Phospholipidosis and Steatosis Detection Kits. Various portions of the procedure may be omitted depending on the investigation undertaken. For example, phospholipid and neutral lipid staining can be performed in sequence (as shown) or as isolated experiments. Nuclear staining may be incorporated during the fixation step if desired by adding Hoechst 33342, which is provided in the kits.

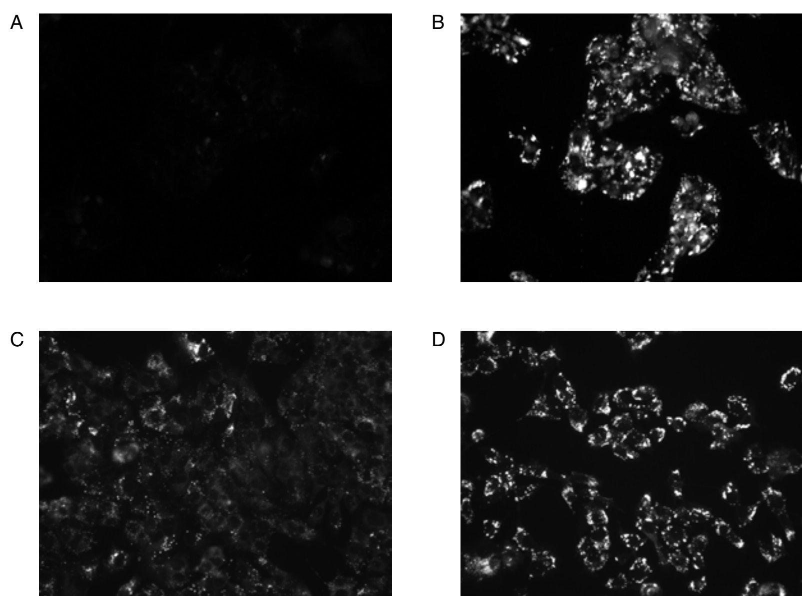


**Figure 2.** Phospholipid and neutral lipid staining in Hep G2 cells. Liver carcinoma cells (Hep G2) were untreated (panels A and C), treated with 30 µM propranolol (panel B), or treated with 30 µM cyclosporin A (panel D) for 48 hours and labeled with LipidTOX™ Red phospholipidosis detection reagent (panels A and B) or LipidTOX™ Green neutral lipid stain (panels C and D) according to the protocol in this manual. Pronounced phospholipid staining is observed in the sample treated with propranolol (panel B) and pronounced neutral lipid staining is observed in the sample treated with cyclosporin A (panel D).

## Before You Begin

Allow all vials to warm to room temperature before opening.

**Caution**

Cyclosporin A (in Cat. nos. H34157, H34158) is a known mutagen. Please handle all of the reagents in the HCS Phospholipidosis and Steatosis Detection Kits using good laboratory practice and dispose of them in accordance with all local regulations.

### Aggregates in Thawed LipidTOX™ Stain Solutions

After thawing LipidTOX™ Red phospholipidosis detection reagent, some minute aggregates might be observed in the solution. They usually will disappear if the vial is incubated in a 37˚C water bath for 5 minutes. These aggregates do not affect the performance of the assay. Any aggregates that remain after the stain is 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/probe 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.

### Using the Positive Control Compounds

**Propranolol**

Propranolol is included as a positive control compound for the induction of phospholipidosis. In Hep G2 cells, 48 hours' incubation with 30 µM propranolol in the presence of LipidTOX™ Red phospholipidosis detection reagent followed by formaldehyde fixation results in very strong intracellular fluorescent signals.

**Cyclosporin A**

Cyclosporin A is included as a positive control compound for the induction of steatosis. In Hep G2 cells, 48 hours incubation with 30 µM cyclosporin A results in reduced cell numbers, enlarged cells, and prominent intracellular lipid droplets. The lipid droplets can be easily labeled with LipidTOX™ Green neutral lipid stain after formaldehyde fixation.

## Experimental Protocols

Volumes given in the protocols below are for cells grown in 96-well plates.

### Phospholipid Staining

**1.1** Prepare the LipidTOX™ Red phospholipidosis detection reagent.

- Dilute the 1000X LipidTOX™ Red 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.
- Filter the 2X stain solution through a 0.2 µm filter certified for cell culture use.

**1.2** Prepare a 2X solution of test compound in normal growth medium. A volume of 50 µL/well is required.

**1.3** **OPTIONAL:** Prepare the **propranolol** positive control compound.

- Make a 10 mM propranolol stock solution using the DMSO provided in the kit. For 35 µg of propranolol (Cat. no. H34157) add 12 µL DMSO to the vial; for 106 µg of propranolol (Cat. no. H34158) add 36 µL DMSO to the vial.
- Vortex vigorously to mix.
- Dilute the 10 mM stock solution of propranolol using normal growth medium to a final concentration of 60 µM. After adding the 10 mM stock solution of propranolol to warm media, mix vigorously to avoid precipitation of the compound. A volume of 50 µL/well is required.

**Note:** Discard unused solutions of 60 µM propranolol in growth medium. The 10 mM DMSO stock solution can be stored at –20˚C for approximately 1 month without significant loss of activity.

**1.4** **OPTIONAL:** Prepare the **cyclosporin A** positive control compound.

- Make a 10 mM cyclosporin A stock solution using the DMSO provided in the kit. For 144 µg of cyclosporin A (Cat. no. H34157) add 12 µL DMSO to the vial; for 432 µg of cyclosporin A (Cat. no. H34158) add 36 µL DMSO to the vial.
- Vortex vigorously to mix.
- Dilute the 10 mM stock solution of cyclosporin A using normal growth medium to a final concentration of 60 µM. After adding the 10 mM stock solution of cyclosporin A to warm media, mix vigorously to avoid precipitation of the compound. A volume of 50 µL/well is required.

**Note:** Discard unused solutions of 60 µM cyclosporin A in growth medium. The 10 mM DMSO stock solution can be stored at –20˚C for approximately 1 month without significant loss of activity.

**1.5** Remove medium from the wells of the microplate.

**1.6** To all wells, add 50 µL/well 2X LipidTOX™ Red phospholipidosis detection reagent (prepared in step 1.1).

**1.7** To the appropriate wells containing LipidTOX™ Red phospholipidosis detection reagent, add 50 µL/well of the growth medium containing the 2X test compound (prepared in step 1.2). The concentration of stain and of test compound in these wells should now be 1X.

**1.8** **OPTIONAL:** To the appropriate wells containing LipidTOX™ Red phospholipidosis detection reagent, add 50 µL/well of the 2X positive control compound (prepared in steps 1.3 and 1.4). The concentration of stain and of control compound in these wells should now be 1X.

**Note:** In the HCS LipidTOX™ Phospholipidosis and Steatosis Detection Kits two positive control compounds are provided. These are intended for use in separate wells, not for combining in the same wells.

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

**Note:** a minimal incubation time of 24 hours is recommended, typically 48 to 72 hours incubation is used.

## Formaldehyde Fixation

The protocol below describes the fixation of cells in the presence of Hoechst 33342 stain. You may omit Hoechst 33342 when fixing cells if nuclear staining is not desired.

**2.1** Prepare formaldehyde/Hoechst 33342 fixative solution.

- Prepare a 3.0–4.0% solution of formaldehyde in buffer. A volume of 100 µL/well is required.
- Add 1000X Hoechst 33342 dye to the diluted formaldehyde solution so that the final volume of Hoechst is 1X. For example, add 12 µL of 1000X Hoechst 33342 to 12 mL of 3.0–4.0% formaldehyde to make 12 mL of fixative solution, enough for 100 wells.
- Mix well.

**2.2** Remove the incubation medium.

**2.3** Add 100 µL of formaldehyde/Hoechst 33342 fixative solution to each well.

**2.4** Incubate for 30 minutes at room temperature.

**2.5** Remove the fixative solution.

**2.6** Rinse the formaldehyde-fixed cells gently with buffer 2–3 times to remove residual formaldehyde. If only labeling phospholipids, the plate can be sealed with plate-sealing film at this stage before analysis. See *Image Acquisition and Analysis*.

**Note:** If neutral lipid staining is desired, proceed to step 3.1.

## Neutral Lipid Staining

The protocol below describes neutral lipid staining for cells grown in 96-well plates. If neutral lipid staining is to be performed without prior phospholipid staining, carry out formaldehyde fixation on the cells (see *Formaldehyde Fixation*, above), then proceed to step 3.1 below.

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

**3.2** Remove buffer from the cells (after the appropriate number of buffer washes from step 2.6).

**3.3** Add 100 µL of 1X LipidTOX™ Green neutral lipid stain to each well.

**3.3** **RECOMMENDED:** Seal the plates with plate-sealing film.

**3.4** Incubate the plates at room temperature for at least 30 minutes before imaging. Image the plate without washing. See *Image Acquisition and Analysis.*

**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 for no more than one week.

The Hoechst 33342–stained nuclei can be used for microscope auto-focusing and serve as a marker for cell identification.

LipidTOX™ Green neutral lipid stain can be imaged with an Alexa Fluor® 488/fluorescein filter set. LipidTOX™ Red phospholipidosis detection reagent is best imaged with an Alexa Fluor® 594/Texas Red® dye filter set.

## Reference


**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 |
|---|---|---|
| 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 |
| 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 |
| 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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