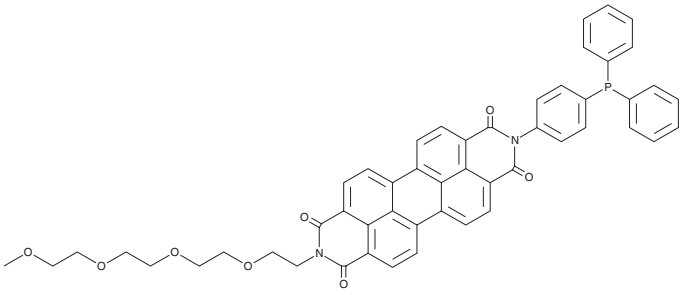
# PRODUCT INFORMATION

## Liperfluo

*Item No. 21467*



**CAS Registry No.:** 1448846-35-2
**Formal Name:** 2-[4-(diphenylphosphino)phenyl]-9-(3,6,9,12-tetraoxatridec-1-yl)-anthra[2,1,9-def:6,5,10-d'e'f']diisoquinoline-1,3,8,10(2H,9H)-tetrone
**MF:** $C_{51}H_{41}N_2O_8P$
**FW:** 840.9
**Purity:** ≥90%
**Ex./Em.:** 488/500-550 nm
**Supplied as:** A crystalline solid
**Storage:** 4°C
**Stability:** ≥1 year
**Special Conditions:** Light sensitive

*Information represents the product specifications. Batch specific analytical results are provided on each certificate of analysis.*

## Description

Liperfluo is a fluorescent probe for lipid peroxides.[1,2] Upon reaction with lipid peroxides, Liperfluo is oxidized and displays an emission maximum of 500-550 nm upon excitation at 488 nm.[1] It is selective for lipid peroxides over hydrogen peroxide, hydroxyl radical, superoxide, nitric oxide, peroxynitrite, alkylperoxyl radical, and cumene hydroperoxide. Monitoring lipid peroxidation has high relevance to various cellular processes, especially ferroptosis, which is an iron-dependent form of cell death characterized by increased lipid peroxidation.[3,4]

## General Protocol

*NOTE: This protocol is provided as a general method. Please optimize the staining conditions, such as the final concentration of Liperfluo and the exposure time of the stimulating agent(s).*

1. Prepare solutions
   a. Prepare a 1 mM stock solution of Liperfluo in DMSO. Dissolve 50 µg of Liperfluo in 60 µl of DMSO and mix by vortexing for approximately 2 minutes.

      *NOTE 1: Protect the Liperfluo stock solution from light and use it within one day.*

      *NOTE 2: If solids are present at the bottom of the tube, extend the vortexing time.*

   b. Prepare a Liperfluo working solution. The concentration of Liperfluo for optimal staining will vary depending on the experimental conditions. Follow the procedures below.

      i. *For microscopic imaging*: Dilute the 1 mM Liperfluo stock solution with serum-free cell culture medium.

      ii. *For flow cytometry*: Add the 1 mM Liperfluo stock solution directly to the cell suspension.



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2. Prepare, label, and image the cells

a. Prepare cells for the assay.

b. Discard the cell culture medium and wash the cells once with serum-free cell culture medium.

c. Add an appropriate volume of the Liperfluo working solution to the cells and incubate at 37°C for 30 minutes.

*NOTE: Optimize the final concentration of Liperfluo as the optimal concentration depends on experimental conditions and other factors.*

d. Discard the working solution and wash the cells twice with serum-free cell culture medium.

e. Observe or analyze the cells using fluorescence microscopy or flow cytometry with excitation and emission wavelengths of 488 and 500-550 nm, respectively.

## Experimental Example

**1. Detection of Lipid Peroxide by Confocal Microscopy**

a. HeLa cells in MEM (containing 10% fetal bovine serum) were seeded ($3.0\times10^4$ cells/well) in a 8 well slide (ibidi) and cultured overnight at 37°C in a 5% $CO_2$ incubator.

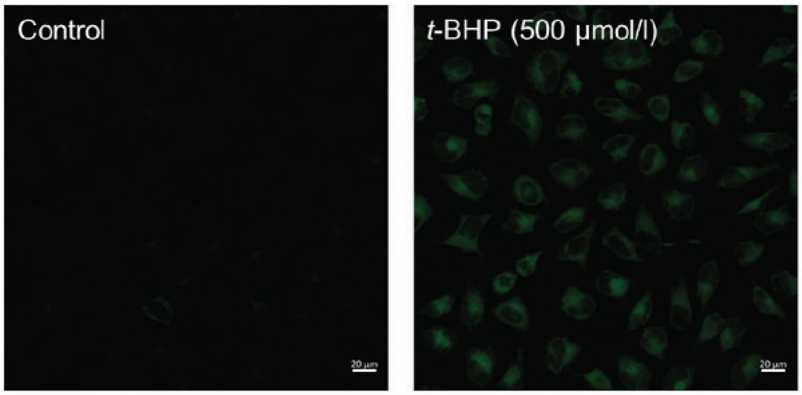
b. Removed the supernatant and the cells were washed with serum-free culture medium once.

c. Serum-free culture medium (200 µl) containing 1 µmol/L Liperfluo was added to well and the cells were incubated at 37°C for 30 minutes in a 5% $CO_2$ incubator.

d. The medium was removed and the cells were washed with 200 µl of HBSS twice.

e. HBSS (200 µl) containing 500 µmol/L t-BHP (tert-buthyl hydroperoxide) was added to the well and the cells were incubated for 60 minutes at 37°C in a 5% $CO_2$ incubator.

f. The cells were observed under a confocal fluorescence microscope. (Ex: 488 nm, Em: 500-550 nm).




Fluorescence images of lipid peroxidation in HeLa cells.

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**2. Detection of Lipid Peroxide by Flow Cytometry**

a. HeLa cells in MEM (containing 10% fetal bovine serum) were seeded ($1.0\times10^5$ cells/well) in a 6 well plate (Thermo) and cultured overnight at 37°C in a 5% $CO_2$ incubator.

b. Removed the supernatant and the cells were washed with serum-free culture medium once.

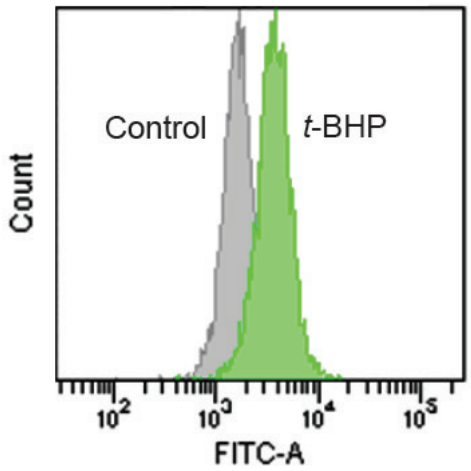
c. Serum-free culture medium (2 ml) containing 1 µmol/L Liperfluo was added to well and the cells were incubated at 37°C for 30 minutes in a 5% $CO_2$ incubator.

d. The medium was removed and the cells were washed with 2 ml of HBSS twice.

e. HBSS (2 ml) containing 500 µmol/L t-BHP (tert-buthyl hydroperoxide) was added to the well, and the cells were incubated for 60 minutes at 37°C in a 5% $CO_2$ incubator.

f. The cells were harvested by trypsinization and collected into a microtube with culture medium.

g. The supernatant was replaced with HBSS and the cells were analyzed using a flow cytometer (Ex: 488 nm, Em: 515-545 nm).




Quantification of lipid peroxide with Liperfluo.

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**3. Detection of Lipid Peroxide Induced by Ferroptosis with Erastin**

a. A549 cells in DMEM (containing 10% fetal bovine serum) were seeded ($1.0\times10^5$ cells/well) in a 8 well slide (ibidi) and cultured overnight at 37°C in a 5% $CO_2$ incubator.

b. The DMEM was removed and 200 µl of serum-free culture medium containing 50 µmol/L erastin was added to well.

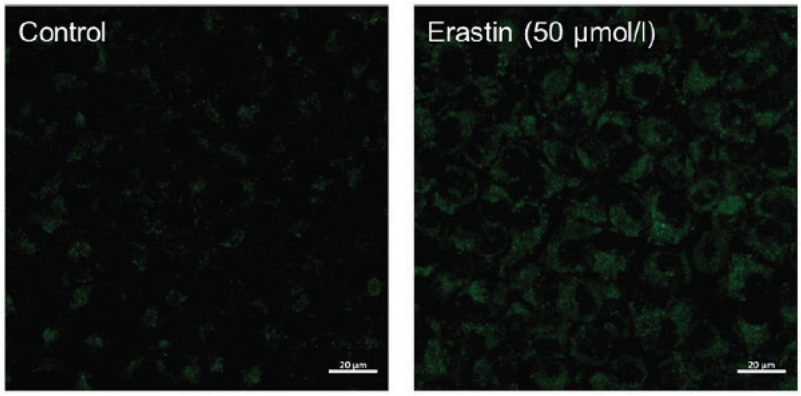
c. The cells were incubated at 37°C overnight in a 5% $CO_2$ incubator.

d. The medium was removed and the cells were washed with 200 µl of HBSS twice.

e. HBSS (200 µl) containing 5 µmol/L Liperfluo was added to well and the cells were incubated at 37°C for 30 minutes in a 5% $CO_2$ incubator.

f. The supernatant was removed and the cells were washed with 200 µl of HBSS twice.

g. The cells were observed under a confocal fluorescence microscope. (Ex: 488 nm, Em: 500-550 nm)




Fluorescence images of lipid peroxidation in A549 cells stimulated by erastin.
Stronger fluorescent signal of Liperfluo was observed in the ferroptosis induced sample.

## References

1. Yamanaka, K., Saito, Y., Sakiyama, J., *et al.* A novel fluorescent probe with high sensitivity and selective detection of lipid hydroperoxides in cells. *RSC Adv*. **20**, 7894-7900 (2012).
2. Kagan, V.E., Mao, G., Qu, F., *et al.* Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis. *Nat. Chem. Biol*. **13(1)**, 81-90 (2017).
3. Angeli, J.P.F., Miyamoto, S., and Schulze, A. Ferroptosis: The greasy side of cell death. *Chem. Res. Toxicol*. **32**, 362-369 (2019).
4. Yang, W.S. and Stockwell, B.R. Ferroptosis: Death by lipid peroxidation. *Trends Cell Biol*. **26(3)**, 165-176 (2016).

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