

General Information

Liperfluo, a Spy-LHP analog, is used for lipid peroxide detection, and it emits intense fluorescence by the lipid peroxide specific oxidation in organic solvents such as ethanol (Fig. 1, 2). Since the excitation and emission wavelengths of the oxidized form of Liperfluo are 524 nm and 535 nm, respectively, both photo-damage against a sample and an auto-fluorescence from the sample can be minimized. Due to the introduction of a tetraethyleneglycol group at the one-end of diisoquinoline ring, the dispersibility of the molecule in an aqueous buffer is improved. Though Liperfluo oxidized form is almost non-fluorescent in an aqueous media, it emits fluorescence in lipophilic sites such as in cell membranes. Therefore it can be easily applied for a lipid peroxide imaging by fluorescence microscopy or lipid peroxide analysis by flow cytometry for a living cell.

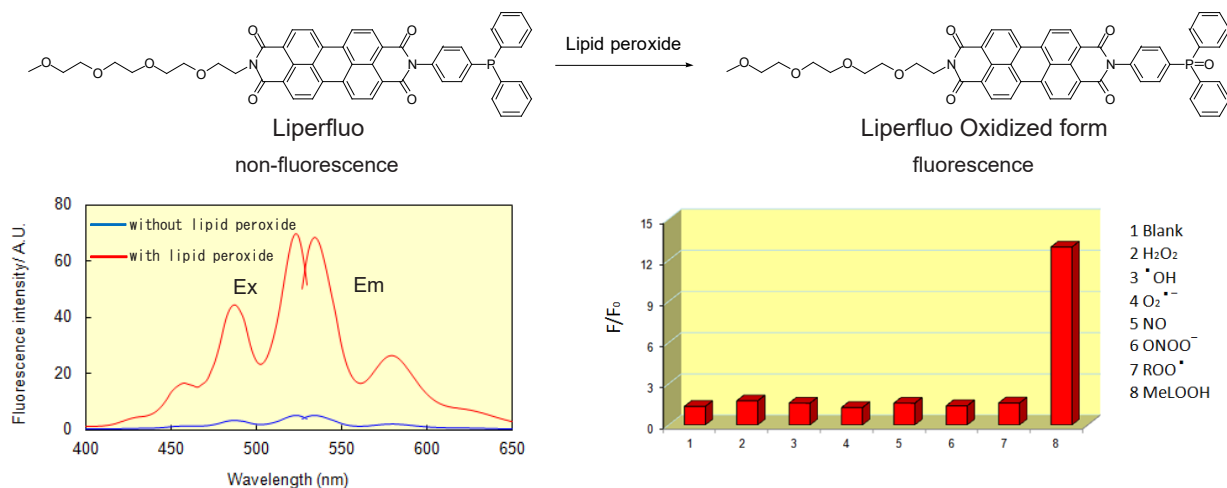


Fig 1. Excitation and emission spectra of Liperfluo with or without lipid peroxide in ethanol.

Fig 2. Reaction selectivity of Liperfluo against to the various reactive oxygen species.

Kit Contents

Liperfluo 50 µg × 5

Storage Conditions

Store at 0-5 °C and protect from light

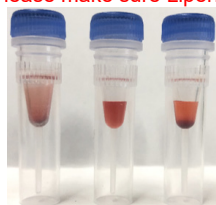
Preparation of Solutions

Preparation of a 1 mmol/l Liperfluo DMSO stock solution

To prepare a 1 mmol/l Liperfluo DMSO stock solution, add 60 µl DMSO to a tube containing Liperfluo and pipette approx. 30 times and vortex the solution to dissolve Liperfluo completely.

*Protect the Liperfluo DMSO stock solution from light with aluminum foil and use it within a day once prepared.

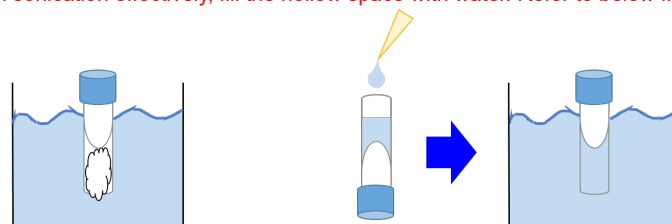
*Please make sure Liperfluo of the inner tube are completely dissolved.



Left image : Liperfluo
Middle image : Liperfluo (completely dissolved)
Right image : Liperfluo (dissolving is not enough)

*Sonication or warming (40 °C, 3 minutes) can be an alternative way to pipetting and vortex process necessary for dissolving.

*To perform sonication effectively, fill the hollow space with water. Refer to below figure.



✗ Air in the hollow space may lower the efficiency of dissolving.

○ Fill the hollow space with water.

For microscopic imaging

Dilute the 1 mmol/l Liperfluo DMSO stock solution with serum-free culture medium to prepare Liperfluo working solution.

*The concentration of Liperfluo for optimal staining will vary depending on the experimental conditions.

For flow cytometry

Add 1 mmol/l Liperfluo DMSO stock solution directly to cell suspension to be analyzed.

*The concentration of Liperfluo for optimal staining will vary depending on the experimental conditions.

General Protocol

Detection of lipid peroxide in the cells

1. Seed the cells in a dish or plate.
2. Remove the supernatant and wash the cells with serum-free culture medium once.
3. Add an appropriate volume of Liperfluo working solution and incubate at 37 °C for 30 minutes.
4. Remove the supernatant and wash the cells with serum-free culture medium twice.
5. Observe the cells under a fluorescence microscope or analyze the cells using a flow cytometer.

Detection of lipid peroxide by confocal microscopy

- (1) HeLa cells in MEM (containing 10% fetal bovine serum) were seeded (3.0×10^4 cells/well) in a 8 well slide (ibidi) and cultured overnight at 37 °C in a 5% CO₂ incubator.
- (2) The cells were washed with serum-free culture medium once.
- (3) 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% CO₂ incubator.
- (4) The medium was removed and the cells were washed with 200 µl of HBSS twice.
- (5) HBSS (200 µl) containing 500 µmol/l *t*-BHP (*tert*-butyl hydroperoxide) was added to the well and the cells were incubated for 60 minutes at 37 °C in a 5% CO₂ incubator.
- (6) The cells were observed under a confocal fluorescence microscope. (Excitation: 488 nm, Emission: 500-550 nm)

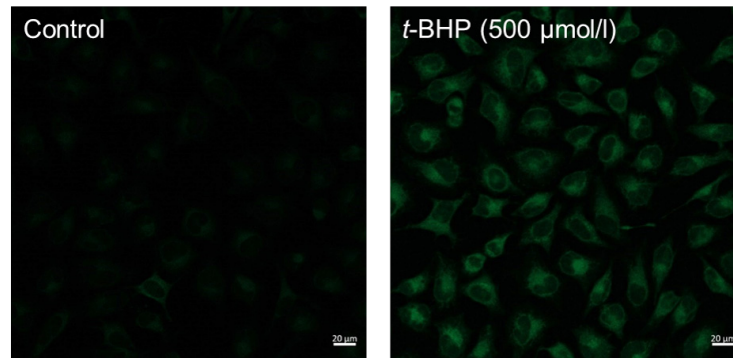


Fig 3. Fluorescence images of lipid peroxidation in HeLa cells.

Detection of lipid peroxide by Flow Cytometry

- (1) HeLa cells in MEM (containing 10% fetal bovine serum) were seeded (1.0×10^5 cells/well) in a 6 well plate (Thermo) and cultured overnight at 37 °C in a 5% CO₂ incubator.
- (2) The cells were washed with serum-free culture medium once.
- (3) 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% CO₂ incubator.
- (4) The medium was removed and the cells were washed with 2 ml of HBSS twice.
- (5) HBSS (2 ml) containing 500 µmol/l *t*-BHP (*tert*-butyl hydroperoxide) was added to the well, and the cells were incubated for 60 minutes at 37 °C in a 5% CO₂ incubator.
- (6) The cells were harvested by trypsinization and collected into a microtube with culture medium.
- (7) The supernatant was replaced with HBSS and the cells were analyzed using a flow cytometer. (Excitation: 488 nm, Emission: 500-550 nm)

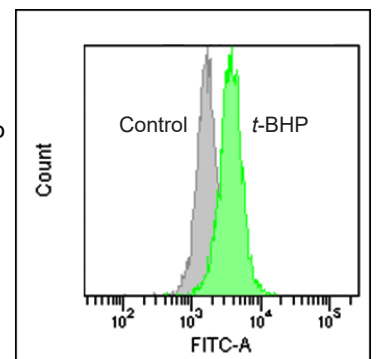


Fig 4. Quantification of lipid peroxide with Liperfluo.

Detection of lipid peroxide induced by ferroptosis with erastin (inhibitor for cystine transporter)

- (1) A549 cells in DMEM (containing 10% fetal bovine serum) were seeded (1.0×10^5 cells/well) in a 8 well slide (ibidi) and cultured overnight at 37 °C in a 5% CO₂ incubator.
- (2) The DMEM was removed and 200 µl of serum-free culture medium containing 50 µmol/l erastin was added to well.
- (3) The cells were incubated at 37 °C overnight in a 5% CO₂ incubator.
- (4) The medium was removed and the cells were washed with 200 µl of HBSS twice.
- (5) 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% CO₂ incubator.
- (6) The supernatant was removed and the cells were washed with 200 µl of HBSS twice.
- (7) The cells were observed under a confocal fluorescence microscope. (Excitation: 488 nm, Emission: 500-550 nm)

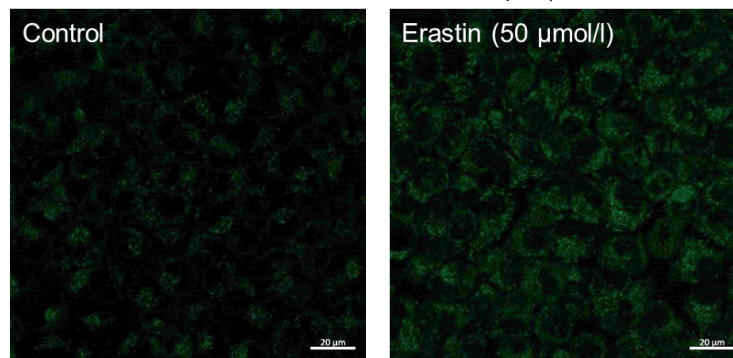


Fig 5. Fluorescence images of lipid peroxidation in A549 cells stimulated by erastin. Stronger fluorescent signal of Liperfluo was observed in the ferroptosis induced sample.

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If you need more information, please contact Dojindo technical service.

Dojindo Laboratories
2025-5 Tabaru, Mashiki-machi, Kamimashiki-gun, Kumamoto
861-2202, Japan Phone: +81-96-286-1515 Fax: +81-96-286-1525
E-mail: info@dojindo.co.jp Web: www.dojindo.co.jp

Dojindo Molecular Technologies, Inc.
Tel: +1-301-987-2667 Web: http://www.dojindo.com/
Dojindo EU GmbH
Tel: +49-89-3540-4805 Web: http://www.dojindo.eu.com/
Dojindo China Co., Ltd
Tel: +86-21-6427-2302 Web: http://www.dojindo.cn/
Tel: +86-10-8225-1765