Detection of lipid peroxide by fluorescent microscopy and flowcytometry

# Liperfluo

**Product Code: L248**

Liperfluo, a perylene derivative containing oligooxyethylene, is designed and exclusively developed by Dojindo for a detection of lipid peroxides and emits intense fluorescence by a lipid peroxide specific oxidation in organic solvents such as ethanol. Among fluorescent probes that detect Reactive Oxigen Species(ROS), Liperfluo is the only compound that can specifically detect lipid peroxides. Since the excitation and emission wavelengths of the oxidized Liperfluo are 524 nm and 535 nm, respectively, both a photo-damage against a sample and an auto-fluorescence from the sample can be minimized. The tetraethyleneglycol group linked to one end of diisoquinoline ring helps its solubility and dispersibility to aqueous buffer. Though Liperfluo oxidized form is almost nonfluorescent in an aqueous media, it emits fluorescence in lipophilic sites such as in cell membranes. Therefore it can easily be applied to lipid peroxide imaging by a fluorescence microscopy and a flow cytometric analysis for living cells.

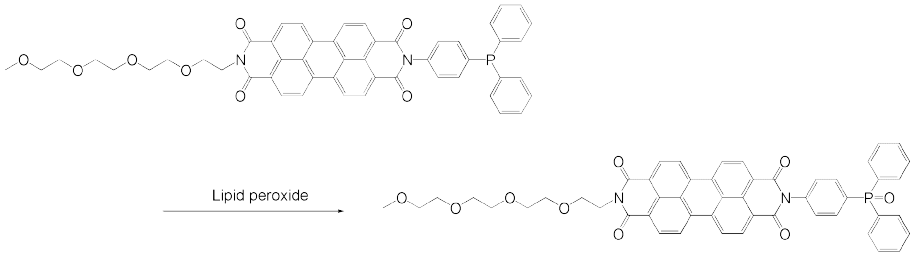



**Fig. 1 Reaction of Liperfluo with lipid peroxide**

## 1. General Protocol

1. Add 60 µl of DMSO to a vial containing Liperfluo(50 µg) and dissolve the product (concentration: 1 mmol/l).
   - If it is hard to dissolve it by pipetting, use vortex mixer, sonicator or warm the solution.
   - Cover the solution with an aluminum foil and use it within a day after the reconstition.
2. Add 10 µl of the Liperfluo solution to 1 ml of cell culture containing 1.0 x $10^5$ cells.
   - Higher final concentration of DMSO may cause damage to cells.
   - Since the background fluorescence may be increased in culture medium, replacing the medium with an aqueous buffer such as PBS is recommended before the addition of Liperfluo solution.
3. Incubate the cell suspension at 37°C for 30 minutes.
4. Analyse the cells with a fluorescence microscope or a flow cytometer.
   - Although the Liperfluo oxidized form is almost non-fluorescent in an aqueous solution, wash the cells with PBS as necessary if the background fluorescence is high.

## 2. Live cell imaging of lipid peroxide (Fig. 4)

1. Innocurate SH-SY5Y cells(6.0 x $10^5$ cells/well) to a 6-well plate.
2. Incubate the plate at 37 ºC for overnight.
3. Add Liperfluo, DMSO solution(final conc. 20 µM) and incubate at 37 ºC for 15 minutes.
4. Add either Cumene Hydroperoxide(final conc. 100 µM) or AIPH*(final conc. 6 mM).
5. Incubate at 37ºC for 2 hours.
6. Observe fluorescent by microscope**(Ex. 524 nm, Em. 535 nm).

* AIPH: 2,2'-azobis-[2-(2-imidazolin-2-yl)propane]dihydrochloride

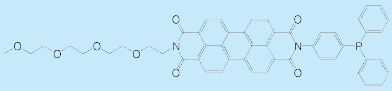
** Olympus IX-71 epifluorescent microscope, mirror unit: U-MNIBA3, exposure time: 10 sec, ISO: 800

## 3. References


1. K. Yamanaka, *et al*., A novel fluorescent probe with high sensitivity and selective detection of lipid hydroperoxides in cells, RSC Advances, 2012, **2**, 7894.
2. N. Soh, *et al*., Novel fluorescent probe for detecting hydroperoxides with strong emission in the visible range. *Bioorg Med Chem Lett*. 2006;**16**:2943-2946.
3. N. Soh, *et al*., Swallow-tailed perylene derivative: a new tool for fluorescent imaging of lipid hydroperoxides. *Org Biomol Chem*. 2007;**5**:3762-3768.


## 4. Specification

- ► Appearance: reddish black crystalline powder
- ► Purity: ≥90.0%(HPLC)



**Liperfluo**

N-(4-Diphenylphosphinophenyl)-N'-(3,6,9,12-tetraoxatridecyl)perylene-3,4,9,10-tetracarboxydiimide

$C_{51}H_{41}N_2O_8P$ = 840.85,

Unit: 50 µg x 5

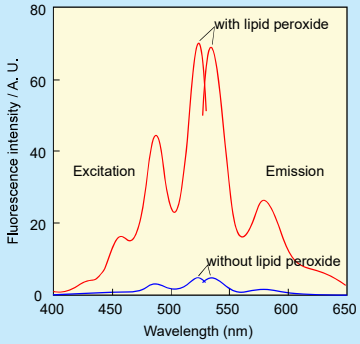



**Fig. 2 Excitation and emission spectra of Liperfluo with or without lipid peroxide in ethanol.**

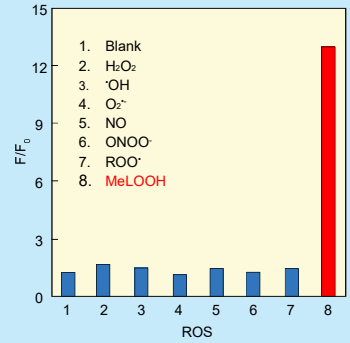



**Fig. 3 Reaction selectivity of Liperfluo against the various reactive oxygen species.**

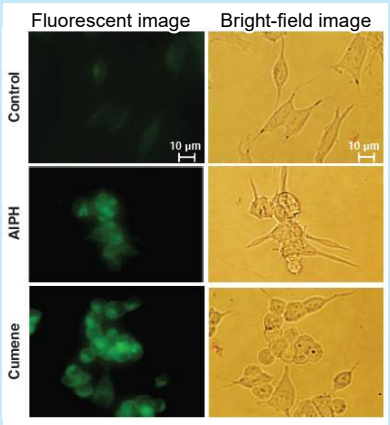



**Fig. 4 Reaction selectivity of Liperfluo against the various reactive oxygen species.**

Data was kindly provided from Dr. N. Noguchi, Doshisha University, System Life Science Laboratory.