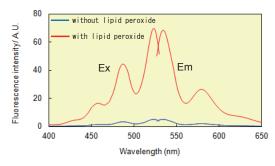
Technical Manual ( Japanese version) is available at http://www.doi.indo.co.in/manual/1248.pdf

## Introduction

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 autofluorescence 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.

non-fluorescence

Liperfluo oxidized form fluorescence



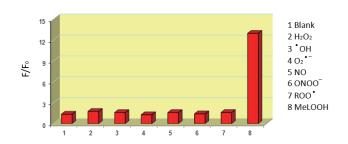


Fig 1. Excitation and emission spectra of Liperfluo with or without lipid peroxide in ethanol.

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

## **Kit Contents**

## Liperfluo 50 µg x 5

## Storage General Protocol

Store at 0-5°C. This reagent is stable for 6 months at 0-5°C after purchase.

Detection of lipid peroxide in cells

- 1. Add 60 µl DMSO to a tube containing Liperfluo (50 µg) and dissolve with pipetting (concentration: 1 mmol/l).
  - Liperfluo is slightly soluble in DMSO by pipetting, therefore use a vortex mixer and a sonicator, or warm the solution to dissolve Liperfluo.
  - Protect the Liperfluo (DMSO) solution with an aluminum foil and use it within a day after preparing the solution because of its light sensitivity.
- 2. Apply the Liperfluo (DMSO) solution to cells.

Ex.) Add an appropriate volume of the Liperfluo (DMSO) solution to 1 ml of cell suspension (1.0 × 10<sup>5</sup> cells/ml).

Volume	Liperfluo conc.
10 μΙ	10 μmol/l
5 µl	5 μmol/l
1 µl	1 µmol/l

- The DMSO concentration should be 1% or lower after the addition to the cell suspension.
- Since the background fluorescence might increase in culture medium, we recommend to replace with an aqueous buffer such as PBS before adding Liperfluo solution.
- 3. Incubate the cell suspension at 37°C for 30 min.
- 4. Analyse the cells with a fluorescence microscope or a flow cytometer.
  - Although Liperfluo oxidized form is almost non-fluorescent in an aqueous solution, wash the cells with PBS as necessary if the background fluorescence is high.

This protocol is a general method. Optimize the conditions for your experiments.

If you require assistance, please contact Dojindo customer service.

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