Lipid Peroxide (LPO) Assay Kit（BODIPY 581/591 C11）

Cat #: KTA9030 Size: 1000 T/5000 T

	Lipid Peroxide (LPO) Assay Kit（BODIPY 581/591 C11）		
REF	**Cat #:** KTA9030	LOT	**Lot #:** Refer to product label
	Applications: Flow cytometry, fluorescence microscopy and microplate reader detection for cell samples		
	Fluorescence Excitation/ Emission: Oxidized: Ex/Em=488/525 nm; Reduced: Ex/Em=581/591 nm (In actua configuration, 530-561 nm excitation can be selected based on the instrument's excitation module.)		
	Note: Stored at -20℃ for 12 months, protected from light		

Assay Principle

Lipid Peroxidation (LPO) is a core marker of damage to biological membrane systems and is widely involved in various pathological processes such as atherosclerosis, neurodegenerative diseases and ischemia-reperfusion injury. Particularly importantly, lipid peroxidation is a key downstream characteristic of Ferroptosis. During ferroptosis, polyunsaturated fatty acids in membrane phospholipids undergo chain oxidation reactions catalyzed by iron ions, leading to membrane structure disintegration and cell dysfunction. Therefore, detecting intracellular lipid peroxidation levels is of great significance for exploring the mechanisms of related diseases and the regulation of ferroptosis.

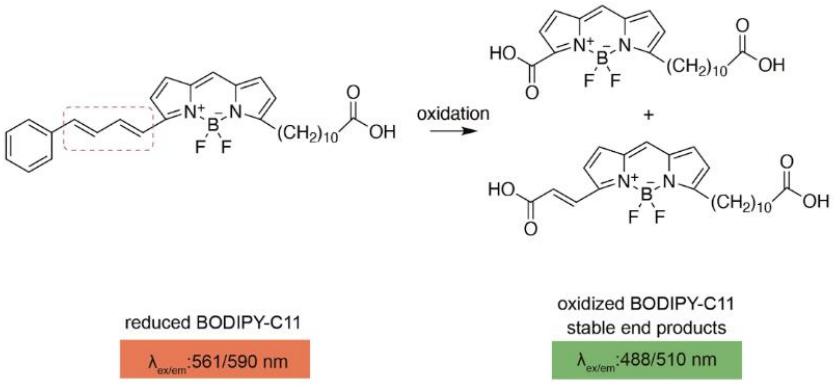


Figure 1. Chemical structural formulas of the BODIPY 581/591 C11 probe before and after oxidation.

This kit adopts the specificity of fluorescent probe BODIPY 581/591 C11, rapid, high sensitive detection lipid peroxidation level in living cells. Under normal circumstances, BODIPY 581/591 C11 is in the reduced state. Its excitation wavelength and emission wavelength are 581/591 nm, and it shows red fluorescence. When the polyunsaturated butadienyl part of BODIPY 581/591 C11 is oxidized, its emission wavelength shifts from 590 nm to 510 nm. At this time, the excitation wavelength and emission wavelength are 488/510 nm, which is green fluorescence. The level of lipid peroxidation can be directly reflected by the change in the ratio of red-green fluorescence. In fact, the increase in lipid peroxide levels can also be characterized by the increase of a single green fluorescence signal. Compared with traditional methods, this probe is not affected by environmental pH and polarity, has strong light stability, low background noise, and is compatible with various detection platforms such as fluorescence microscopes, flow cytometers, and microplate readers. It is an ideal tool for studying lipid peroxidation and ferroptosis.

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Materials Supplied and Storage Conditions

Kit components	Size		storage condition
	1000 T	5000 T	
BODIPY 581/591 C11	1 vial ×2	1 vial ×10	-20℃, protected from light
DMSO	110 μL	550 μL	-20℃, protected from light
LpoUP（1,000×）	60 μL	300 μL	-20℃, protected from light

Note: When the 1000T / 5000T specifications of this kit are used in fluorescence microscopes, fluorescence microplate readers and laser confocal microscopes, taking a 96-well plate as an example, with a 100 μL system per well, 1000 and 5000 detections can be conducted respectively. Taking a 24-well plate as an example, with a 200 μL system per well, 500 and 2,500 tests can be conducted respectively, and so on. When used in flow cytometry, a 200 μL system for each sample can be tested 500 times and 2,500 times respectively.

Materials Required but Not Supplied

·Centrifuge, flow cytometry，fluorescence microscopy or microplate reader
·Precision pipettes, disposable pipette tips, HBSS or Serum-free and phenol red-free medium
·Cell culture plate

Reagent Preparation

Note: The assay buffer used in the probe loading stage and subsequent experiment can be serum-free and phenol red-free basic medium (serum-free medium containing phenol red may increase the background signal), or commercial PBS, DPBS or HBSS can be used. Abbkine CheKine™ Cell Assay Buffer (Cat #: KTB1914) is recommended as a priority. Compared with conventional buffers such as PBS, this buffer has a smaller impact on the morphology of adherent cells and can achieve a better signal-to-noise ratio. After the probe is loaded, the drug and the positive control reagent LpoUP should be prepared with serum-free assay buffer for treatment.

BODIPY 581/591 C11 probe working solution: For the first use, the BODIPY 581/591 C11 powder needs to be centrifuged to the bottom of the tube, and 50 μL of DMSO should be added to each tube to obtain 2 mM probe stock solution, which can be used for subsequent experiments or stored at -20℃ (2 months) or -80℃ (1 year). The working solution is prepared and used immediately: 1 mL of assay buffer +1 μL of probe stock solution (2 mM), until the final concentration is 2 μM. The working solution should be prepared and used immediately and must not be stored.
LpoUP（1,000×）: Dilute 1,000 times to 1x with the assay buffer. Prepare and use immediately. Do not store after dilution.

Assay Procedure

A. Detection by Fluorescence Microscopy

1. For adherent cells

(1) The day before the test, lay out the cell plates to ensure that the cell density reaches about 70% during the test.
(2) Pre-warm the assay buffer at 37℃ for 30 minutes . (Preheating at 37℃ can prevent cells from undergoing morphological changes due to cold stimulation.)
(3) Probe working solution: Dilute BODIPY 581/591 C11 in pre-warmed assay buffer at 37℃ to a final concentration of 2 μM.
(4) Probe loading: in 37℃ preheat assay buffer washing cell 1 to 2 times, add an appropriate volume of the diluted probe working solution. Incubate the cells in a 37℃ incubator away from light for 20-30 min. The probe working solution must

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completely cover the cells.

Reagents	96-well plate	48-well plate	24-well plate	12-well plate	6-well plate
Probe working solution (μL)	100	150	200	400	1000

(5) Remove the probe working solution and wash the cells 1-2 times with assay buffer preheated to 37℃ to thoroughly remove any probes that have not entered the cells.

(6) Drug induction: Remove the wash solution, dilute the drug with assay buffer preheated to 37℃ (serum-free) to stimulate the cells, and incubate the cells in a 37℃ cell incubator protected from light; the actual induction duration depends on the drug properties and the cell type.

(7) Positive control (optional): In step 6, dilute LpoUP (1,000×) in assay buffer to 1× and add it to the cells (no less than 500 μL for 24-well plates, no less than 1 mL for 12-well plates, no less than 2 mL for 6-well plates, and so on); incubate the cells in a 37℃ cell incubator protected from light for approximately 4 h. (The stock concentration of the LpoUP positive control is 50 mM; a recommended final induction concentration of 50 μM is achieved by a 1:1,000× dilution; generally, an induction period of about 4 h is sufficient for Jurkat and HeLa cells; however, the induction time may be extended or shortened depending on the cell type.)

(8) Select an appropriate time point and perform detection using a fluorescence microscope. (It is recommended to conduct a preliminary experiment at several time points to identify the optimal time point for detection.)

Note: Strong laser irradiation may increase the background signal of the NC group. It is recommended to first find the field of view in the bright field, then switch to the FITC channel for detecting the oxidation state, and then switch to the TxRed channel for detecting the reduction state.

2. For suspension cells

Staining and sample preparation were carried out according to the suspended cell operation in the flow cytometry sample, and then transferred to a well plate or glass slide for fluorescence Microscopy examination.

B. Detection by Fluorescence Microscopy

1. For adherent cells

The staining and sample preparation procedure is the same as that for staining adherent cells in the fluorescence microscope: after induction, wash the cells once with a buffer such as PBS (note that any floating cells that appear during induction should also be collected by centrifugation and pooled); then digest the cells with a mild digestive solution, allowing direct flow cytometry analysis within a short period (note that residual enzymes are still active during digestion, so the process must be completed quickly); alternatively, add serum-containing medium to terminate digestion, quickly replace it with serum-free buffer and rinse thoroughly, then resuspend the cells in PBS for flow cytometry analysis.

2. For suspension cells

(1) Collect the cells and prepare a cell suspension; it is recommended that, when loading the probe and inducing LpoUP, the cell density be approximately 0.5-2.0×10^6 cells/mL.

(2) Pre-warm the assay buffer at 37℃ for 30 minutes .

(3) Probe working solution: Dilute BODIPY 581/591 C11 in pre-warmed assay buffer at 37℃ to a final concentration of 2 μM.

(4) Probe loading: Collect the cells and wash them 1-2 times with assay buffer preheated to 37℃, add the diluted probe so that the cell density reaches 0.5-2.0 × 10^6 cells/mL, and incubate the cells in a 37℃ incubator away from light for 20-30 min.

(5) Centrifuge, remove the supernatant and wash the cells 1-2 times with 37℃ preheated assay buffer to thoroughly remove any probes that have not entered the cells.

(6) Drug induction: Remove the wash solution, dilute the drug with assay buffer preheated to 37℃ (serum-free) to stimulate the cells, and incubate the cells in a 37℃ cell incubator protected from light; the actual induction duration depends on the drug properties and the cell type.

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(7) Positive control (optional): In step 6, dilute LpoUP (1:1,000×) with assay buffer to 1×, add it to the cells (to achieve a cell density of approximately 1×10⁶ cells/mL), and incubate in a 37℃ cell culture incubator protected from light for approximately 4 h. (The stock concentration of the LpoUP positive control is 50 mM; a recommended final induction concentration of 50 μM is achieved by a 1:1,000× dilution; generally, an induction period of about 4 h is sufficient for Jurkat and HeLa cells; however, the induction time may be extended or shortened depending on the cell type.)

(8) Select an appropriate time point and perform flow cytometry analysis on the cells. (It is recommended to conduct a preliminary experiment at multiple time points to identify the optimal time point for detection.)

Note: The FITC channel detects the oxidized state, while the PE channel detects the reduced state.

C. Detection by fluorescence microplate reader

1. For adherent cells

Spread the cells onto a 96-well plate, stain them according to the sample preparation procedure for adherent cells described in the fluorescence microscope manual, and then detect them using a fluorescence microplate reader.

2. For suspension cells

Prepare and stain the suspension cells according to the protocol for flow cytometry; then, transfer 100 μL of the cell suspension into each well of a 96-well fully black or fully black-bottom transparent plate for detection.

Note: For the green channel, use 488 nm as the excitation wavelength and 525 nm as the emission wavelength; for the red channel, use 530-561 nm for excitation and 591 nm for detection. (It is recommended to conduct a preliminary experiment at several time points to identify the optimal time point for detection.)

Typical Data

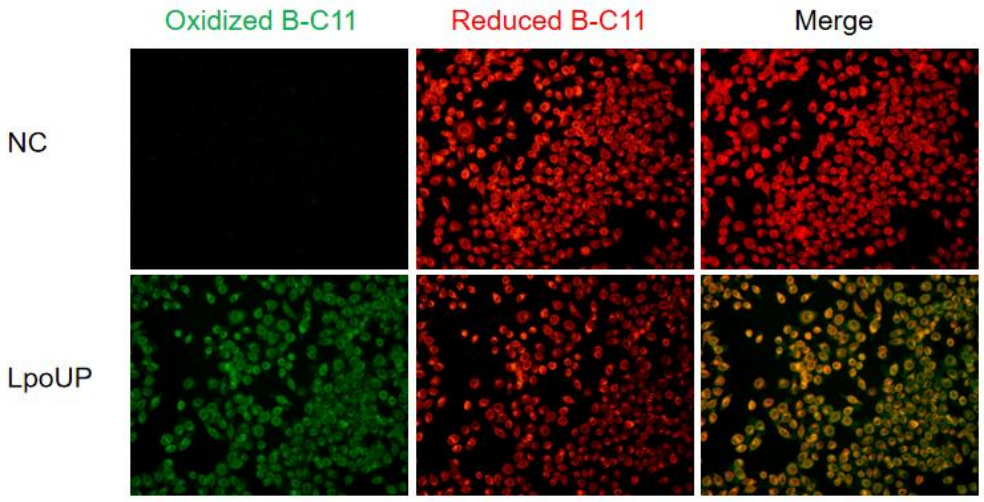


Figure 2. After loading HeLa cells with the BODIPY 581/591 C11 probe (Cat #: KTA9030), the cells were induced with LpoUP for 4 h and then examined by fluorescence microscopy.

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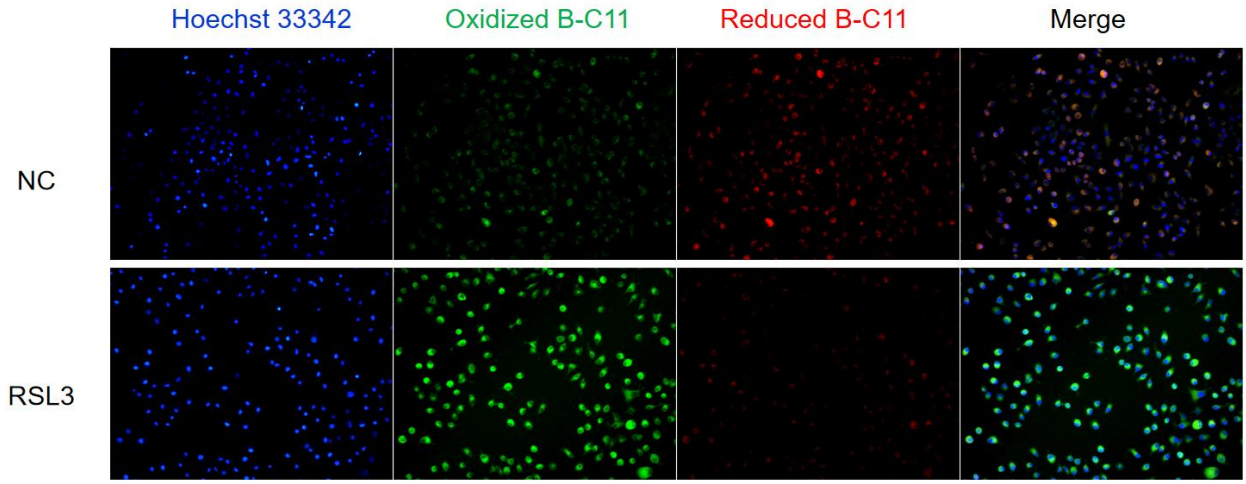


Figure 3. A549 cells were treated with 10 μM RSL3 for 24 h, stained with the BODIPY 581/591 C11 probe (Cat #: KTA9030) and Hoechst 33342, and examined by fluorescence microscopy.

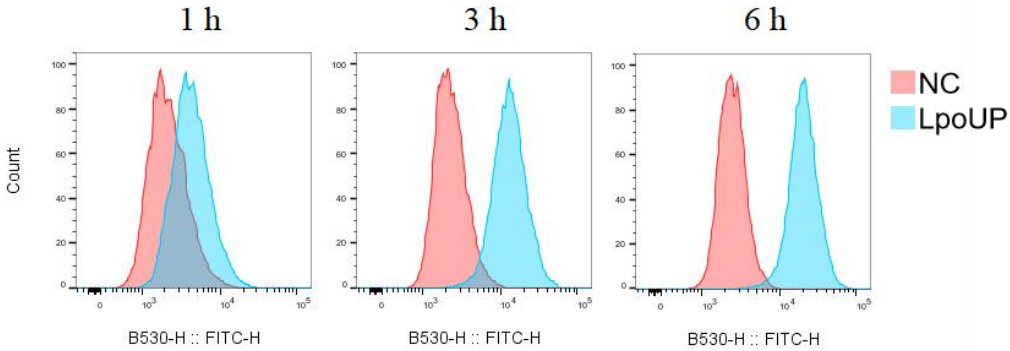


Figure 4. Jurkat cells loaded with the BODIPY 581/591 C11 probe (Cat #: KTA9030) were stimulated with LpoUP (1:1,000) at different time points and analyzed by flow cytometry.

Precautions

1. During the experiment, it is recommended to detect the green signal before the red signal; green fluorescence progresses from no signal to detectable levels, whereas red fluorescence decreases from a strong signal; an increase in green fluorescence alone indicates that oxidation has occurred, allowing the green signal to be used independently for comparison. Some cells have a larger volume, resulting in a strong background red signal; therefore, it is normal for the red signal not to decrease when red fluorescence appears. Commonly used flow cytometer the same excitation lasers for FITC and PE; in addition to effects such as crosstalk, this can lead to an increase in green fluorescence without a corresponding decrease in red fluorescence, or a simultaneous increase in both. It is therefore recommended to compare only the green channel signals; the same principle applies to fluorescence microscopy.
2. Before loading the probe, cells must be thoroughly washed, as serum significantly reduces probe signal; if the induction process after probe loading contains serum, the probe signal will likewise be significantly reduced.
3. For your safety and health, please wear a lab coat and wear disposable gloves.

FAQ

1．Recommendations for fluorescence microscope imaging?

First, select an appropriate observation field before entering the light field; it is recommended to acquire images of the green channel first, followed by images of the red channel. Please note: prolonged exposure to intense laser radiation can readily increase the background signal in the negative control group (NC group).

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2．How should a strong solution background signal be handled during fluorescence microscope imaging?

Replace the solution with fresh assay buffer before imaging, or adjust the instrument's contrast to perform background subtraction.

3．How should the situation be handled if no positive signal is detected after staining?

It is recommended to complete probe loading first, followed by the induction experiment; the probe-loading stage and the entire period from loading until detection should be maintained in a serum-free environment, as serum can quench the probe's fluorescence signal.

4．How should changes in the morphology of control-group cells be handled during microscope imaging?

It is recommended to use a serum-free, phenol-free medium suitable for cell culture (phenol may increase background) or Abbkine CheKine™ Cell Assay Buffer (Cat #: KTB1914).

Recommended Products

Catalog No	Product Name
KTA9012	Mitochondrial Superoxide Assay Kit with MitoNeoD
KTB9060	CheKine™ Pro Cell Ferrous Ion (Fe^{2+}) Fluorometric Assay Kit
KTB1910	CheKine™ Reactive Oxygen Species (ROS) Detection Fluorometric Assay Kit (Green Fluorescence)
KTB1913	CheKine™ Reactive Oxygen Species (ROS) Detection Fluorometric Assay Kit (Red Fluorescence)
KTB1052	CheKine™ Plus Micro Malondialdehyde (MDA) Content Assay Kit
KTB1914	CheKine™ Cell Assay Buffer

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