AB308325 – Lipid Peroxidation Assay Kit (Cell-based)

Screening for potential molecules for induction or inhibition of lipid peroxidation.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: Abcam Website

Storage and Stability

Upon arrival, store the entire kit at -20 °C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

Materials Supplied

Item	Quantity	Storage Condition
Wash Buffer (10X)	25 ml	4 °C
Fixative Solution	10 ml	-20°C
Permeabilization Buffer (10X)	25 ml	4 °C
Cumene Hydroperoxide	20 µl	-20°C
Linoleic Acid Label (500X)	20 µl	-20°C
Copper Reagent (100X)	100 µl	-20°C
Fluorescent Azide (100X) (Avoid light)	100 µl	-20°C
Reducing Agent (20X)	500 µl	-20°C
Total DNA Stain (1000X) (Avoid light)	20 µl	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Tissue culture vessels and appropriate culturing media
- Phosphate Buffered Saline (PBS, pH 7.4)
- Sterile 0.1% Gelatin Solution (optional, only required for suspension cells)
- Flow cytometer equipped with laser capable of excitation at 488 nm wavelength (FL-1)
- Fluorescence microscope capable of excitation and emission at 440/490 nm

Reagent Preparation

10X Wash Buffer and 10X Permeabilization Buffers: Thaw at 37 °C to dissolve completely. Dilute the 10X stocks 1:10 in sterile water, mix well. Store at 4 °C.

Fixative Solution: Divide into aliquots and store at -20°C, protected from light.

Cumene Hydroperoxide: Provided as a stock solution (50000X). Divide into aliquots and store at -20 °C, protected from light. To make 100X working solution, mix 2 µl of Cumene Hydroperoxide stock solution with 998 µl PBS, immediately prior to use.

Remaining Components: Store at -20 °C, protected from light. While in use, keep on ice and minimize light exposure.

Lipid Peroxidation Assay Protocol:

Δ Note: *This assay was developed using HeLa (adherent) and Jurkat (suspension) cells and can be modified for any cell line. The protocol below refers to a 96-well tissue culture plate. Adjust the volume accordingly for other plate formats. The assay volume is 100 µl. Growth conditions, cell number per well and other factors may affect the incorporation rate of the Lipid Label. Therefore, optimize the assay for your cell type. We suggest an initial test of several Linoleic Acid Label concentrations to find the best conditions for your experimental design. Avoid stressing the cells by washes or temperature changes prior to incubation with Linoleic Acid Label. All steps should be carried out at room temperature (RT) unless otherwise specified. Equilibrate all buffers to RT prior to the experiment.*

Preparation of Control and Experimental Cells:

1. For high resolution microscopy: To immobilize suspension cells for microscopy, add 100 µl of 0.1% Gelatin solution directly into the wells, tilt the plate to cover. Obtain cell suspension, and seed directly into tissue culture vessels (Hela cells (10^6 cells/ml) and Jurkat ($5x10^6$ cells/ml)), or on coverslips the entire well surface, place it in a tissue culture hood for 1 hour. Gently remove the Gelatin solution and seed your cells. Allow the cells to recover overnight before the treatment. Prepare wells with cells that will be treated (Experimental Wells) and 3 controls including a) Negative Control cells (FACS setup control cells, no treatment, and no label; b) Positive Control cells (cumene hydroperoxide and linoleic acid treatment and reaction); c) Background Control cells (reaction only).
2. Next day, for adherent cells, remove the medium directly. For suspension cells, centrifuge cells at 300 x *g* at RT for 5 min and discard the supernatant and replace with fresh medium. Add the Linoleic Acid Label (500X) diluted to 1X final concentration to both the experimental and Positive Control cells and incubate at 37 °C in a 5% CO_2 atmosphere incubator for 30 min. Do not add the Linoleic Acid Label into the Negative Control Cells and Background Control Cells. Following addition of Linoleic Acid Label, for Experimental Cells, add the test compound of interest according to your protocol. For Positive Control Cells, add cumene hydroperoxide (freshly-made 100X working solution) diluted to 1X final concentration with culture medium and incubate at 37 °C in a 5% CO_2 atmosphere incubator for 2 hr.
3. To terminate the experiment for adherent cells, remove the culture media. Wash the cells once with 100 µl PBS, and aspirate the PBS. For suspension cells, centrifuge the cells at 500 x *g* at RT for 5 min. Discard the supernatant and wash the cells once with 100 µl PBS. Centrifuge once again at 500 x *g* at RT for 5 min and aspirate the PBS. Proceed to the Fixation and Permeabilization step.

Δ Note: *For FACS Analysis cells, prior to the Fixation Step, remove the cells from the plate using trypsin or another cell dissociation solution. Collect the adherent cells in microcentrifuge tubes, add equal amounts of complete media to neutralize the trypsin/dissociation solution. Spin at 500 x g at RT for 5 min, and remove the supernatant.*

Fixation and Permeabilization:

1. For adherent cells: Add 100 µl of Fixative Solution to each well and incubate the cells for 15 min at RT, protected from light. Remove the fixative and wash the cells once with 100 µl of 1X Wash Buffer and remove the wash. Add 100 µl of 1X Permeabilization Buffer and incubate the cells for 10 min at RT. Proceed to Linoleic Acid reaction and total DNA staining.
2. For suspension cells: Re-suspend the cells in 100 µl of Fixative Solution and incubate for 15 min at RT, protected from light. Centrifuge the cells at 500 x *g* at RT for 5 min and remove the fixative and wash the cells once with 100 µl of 1X Wash Buffer. Centrifuge the cells at 500 x *g* at RT for 5 min and discard the supernatant and re-suspend the cells in 100 µl of 1X Permeabilization Buffer. Incubate the cells for 10 min at RT. Centrifuge the cells at 500 x *g* and RT for 5 min. Remove the supernatant and proceed to Linoleic Acid reaction and total DNA staining.

Lipid Peroxidation Reaction and DNA Staining:

1. Reaction cocktail: Prepare 1X Reaction Cocktail according to the table below.

Volumes should be multiplied by number of samples and reagents added in the exact order. Use the reaction cocktail within 15 min of preparation.

Δ Note: *Cells should be protected from light during, and following the reaction and DNA staining.*

	Amount per Reaction
PBS	93 µl
Copper Reagent (100X)	1 µl
Fluorescent Azide (100X)	1 µl
Reducing Agent (20X)	5 µl

2. Lipid Peroxidation Reaction: For Negative Control Cells: Add 100 µl of 1X PBS. For Background Control Cells, Positive Control Cells, and Experimental Cells: Add 100 µl of 1X Reaction Cocktail to each sample and incubate the cells for 30 min at RT, protected from light. Remove the Reaction Cocktail and wash cells two times in 500 µl of 1X Wash Buffer by centrifuging the cells each time at 500 x g at RT for 5 min. Remove the 1X wash and suspend the cells in 500 µl of 1X Wash Buffer for FACS analysis.
3. DNA Staining: Prepare 1X dilution of Total DNA Stain and add 100 µl per well. Incubate the cells for 20 min at RT, or at 4 °C overnight, protected from light. Remove the DNA stain solution and wash the cells twice with 500 µl 1X Wash Buffer.

Δ Note: C*ells are compatible with all methods of slide preparation including wet mount or prepared mounting media.*

Fluorescence Microscope Imaging:

Analyze samples for green fluorescence generated by labeled Linoleic Acid and for blue fluorescence by nuclear DNA.

Technical Support

For all technical or commercial enquiries please go to:
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