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ab273282 Palmitoylated Protein Activity Assay Kit (Green)

View Kit datasheet: https://www.abcam.com/ab273282
(use https://www.abcam.cn/ab273282 for china, or
https://www.abcam.co.jp/ab273282 for Japan)

For labeling and detection of palmitoylated proteins.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Table of Contents

1. Overview

Palmitoylated Protein Activity Assay Kit (Green) (ab273282) is a highly specific, simple and robust method for labeling and detection of palmitoylated proteins. The kit uses a modified Palmitic Acid that is fed directly into the cells and gets incorporated into proteins during or post translation. This post translational modification can be followed by click reaction with an azide-containing dye. The assay kit offers a powerful method for imaging localization, trafficking, and dynamics of Palmitoylated proteins or detection by FACS for quantitative studies.

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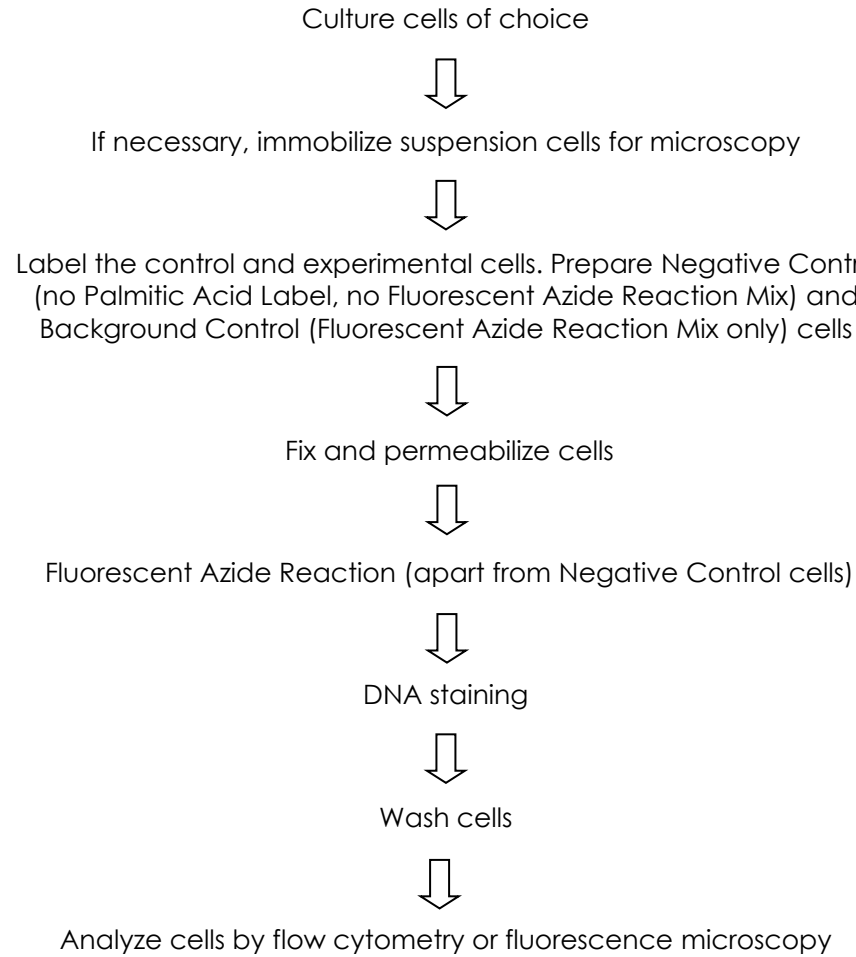
2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
10X Wash Buffer IV/Wash Buffer (10X)	25 mL	-20°C
Fixative Solution I/Fixative Solution	10 mL	-20°C
10X Permeabilization Buffer/Permeabilization Buffer (10X)	25 mL	-20°C
Palmitic Acid Label/Palmitic Acid Label (1000X)	10 µL	-20°C
100X Copper Reagent/Copper Reagent (100X)	100 µL	-20°C
Fluorescent Azide II/Fluorescent Azide (100X)	100 µL	-20°C
20X Reducing Agent/Reducing Agent (20X)	500 µL	-20°C
1000X DAPI/Total DNA Stain (1000X)	20 µL	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform 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 480 nm wavelength (FL-1)
- Fluorescence microscope capable of excitation and emission at 440/490 nm and UV filter

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.
- Hot plate/dry heat block or microplate incubator

9. Reagent Preparation

Briefly centrifuge small vials prior to opening.

9.1 10X Wash Buffer IV/10X Wash Buffer:
Thaw at 37°C to dissolve completely. Dilute the 10X stocks 1:10 in sterile water, mix well. Store at 4°C.

9.2 10X Permeabilization Buffer:
Thaw at 37°C to dissolve completely. Dilute the 10X stocks 1:10 in sterile water, mix well. Store at 4°C.

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

All other reagents should be stored at -20°C protected from light. While in use, keep on ice and minimize light exposure.

10. Assay Protocol

Δ Note: This assay was developed using HeLa (adherent) and Jurkat (suspension) cells and can be modified for any cell lines. The protocol below refers to a 96-well tissue culture plate. Adjust volumes 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 Protein Label. Therefore, optimize the assay for your cell type.

We suggest an initial test of several Palmitic Acid Label concentrations to find the best condition for your experimental design. Avoid stressing the cells by washes or temperature changes prior to incubation with Palmitic Acid Label. All steps should be carried out at room temperature (RT) unless otherwise specified. Equilibrate all buffers to RT prior to the experiment.

10.1 Labeling of control and experimental cells: method with drug pre-incubation:

10.1.1 Obtain cell suspension of desired density and seed directly into tissue culture vessels, or on coverslips for high resolution microscopy

To immobilize suspension cells for microscopy

10.1.2 Add 100 µl of 0.1% gelatin solution directly into the wells.
10.1.3 Tilt the plate to cover the entire well surface and place it in a tissue culture hood for 1 hour.
10.1.4 Gently remove the gelatin solution and seed your cells. Allow the cells to recover overnight before the treatment.
10.1.5 Next day, treat the cells with appropriate effectors according to your protocol.
10.1.6 Do not add treatment to the positive and negative control cells

Negative Control Cells

10.1.7 Unstained cells, cells not exposed to Palmitic Acid Label or Fluorescent Azide II/Fluorescent Azide.

Background Control Cells

10.1.8 Cells are not exposed to the Palmitic Acid Label, only to the Fluorescent Azide II/Fluorescent Azide Reaction.

Positive Control Cells

10.1.9 Cells are incubated with 1X Palmitic Acid Label and Fluorescent Azide II/Fluorescent Azide Reaction.

10.1.10 The next day, for **Adherent cells**, remove the media directly. **For suspension cells**: centrifuge cells at 500 x *g* for 5 minutes and discard the supernatant. Then replace it with fresh aliquots containing Palmitic Acid Label (1000X) diluted to 1X final concentration with culture medium and add into the experimental and positive control cells respectively.

Δ Note: Do not add Palmitic Acid Label to the Negative Control Cells.

10.1.11 Add treatments and incubate the cells for additional 1 day or for the period of time required by your experimental protocol.

Note: Do not remove the drug containing media while incubating with 1X Palmitic Acid Label to avoid potential reversibility of drug action on label incorporation

10.1.12 To terminate the experiment, for **adherent cells**, remove the media and rinse the cells once with 100 µL of 1X PBS, discard the supernatant. **For suspension cells**, centrifuge the cells at 500 x *g* for 5 minutes to deposit the cells onto the surface. Tilt the plate and gently remove the media with a pipette tip.

10.1.13 Make note of the place that is used and perform subsequent aspirations from the same place. Pellet the suspension cells at 500 x g for 5 min throughout the entire protocol.

Δ Note: Avoid excessive centrifugation speed and repeated cycles, which could induce cell damage.

10.2 Fixation and Permeabilization

10.2.1 For adherent cells:

10.2.1.1 Add 100 µL of Fixative Solution I/Fixative Solution to each well and incubate the cells for 15 min at RT, protected from light.

10.2.1.2 Remove the fixative and wash the cells once with 100 µL of 1X Wash Buffer IV/Wash Buffer, remove the wash.

10.2.1.3 Add 100 µL of 1X Permeabilization Buffer and incubate the cells for 10 min at RT.

10.2.1.4 Remove the supernatant and replace it with a 20 µL of 1X Permeabilization Buffer

10.2.2 For suspension cells:

10.2.2.1 Re-suspend the cells in 100 µL of Fixative Solution I/Fixative Solution and incubate for 15 min at RT protected from light. Centrifuge the cells at 500 x *g* for 5 minutes, remove the fixative and wash the cells once with 100 µL of 1X Wash Buffer IV/Wash Buffer.

10.2.2.2 Centrifuge the cells at 500 x g for 5 minutes, discard the supernatant and re-suspend the cells in 100 µL of 1X Permeabilization Buffer. Incubate the cells for 10 min at RT.

10.2.2.3 Centrifuge the cells at 500 x *g* for 5 minutes.

10.2.2.4 Remove the supernatant and replace it with a 20 µL of Permeabilization Buffer. Proceed to Palmitic Acid reaction and total DNA staining.

10.3 Palmitic Acid reaction and total DNA staining:

Reaction Cocktail

10.3.1 Prepare 1X Fluorescent Azide II/Fluorescent Azide Reaction Cocktail according to the table below.

10.3.2 Volumes should be multiplied by number of Samples and reagents added in the exact order.

10.3.3 Use the Fluorescent Azide II/Fluorescent Azide Reaction Cocktail within 15 minutes of preparation.

10.3.4 Cells should be protected from light during and following the reaction and DNA staining.

Component	Amount per reaction
PBS	93 µL
100X Copper Reagent/Copper Reagent (100X)	1 µL
Fluorescent Azide II/Fluorescent Azide (100X)	1 µL
20X Reducing Agent/Reducing Agent (20X)	5 µL

Palmitic Acid Reaction:

10.3.5 For Negative Control Cells, add 100 µL of 1X PBS.

10.3.6 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 30min at RT protected from light.

10.3.7 Remove the Reaction Cocktail and wash cells three times in 100 µL of 1X Wash Buffer IV/Wash Buffer.

10.3.8 Remove the 1X wash and suspend the cells in 100 µL of 1X PBS. Proceed to DNA staining.

10.3.9 If no DNA staining is desired, proceed to Microscopic or FACS analysis.

DNA staining:

10.3.10 Prepare 1X dilution of DAPI/Total DNA Stain and add 100 µL per well.

10.3.10 Incubate the cells for 20 minutes at RT, or refrigerate at 4°C protected from light.

10.3.11 Remove the DNA stain solution. Wash the cells once with 100 µL PBS

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

10.4 Flow Cytometry:

10.4.1 Harvest cells by preferred method and wash with 0.5 mL of ice-cold PBS.

10.4.2 Resuspend pellets with 100 µL of ice-cold PBS and analyze Samples for Green fluorescence generated by Palmitic Acid Label addition during the labeling reaction

10.5 Fluorescence Microscope Imaging:

10.5.1 Analyze Samples for green fluorescence generated by Palmitic Acid Label and for blue fluorescence by nuclear DNA.

11. Typical Data

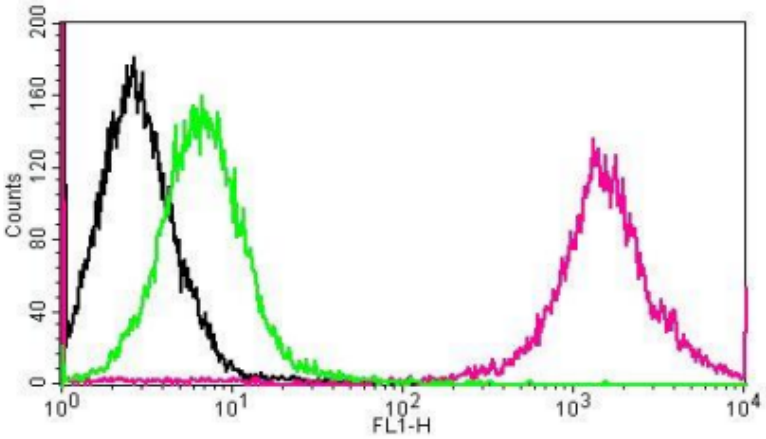


Figure1: Metabolic labeling of palmitic acid on proliferating Jurkat cells. 1 x 10^6 cells incubated overnight with fresh aliquots of media containing Palmitic Acid Label. Analysis of Negative Control (Black), Background (Fluorescent Azide II/Fluorescent Azide only, Green), Positive Control (Palmitic Acid Label and Fluorescent Azide II/ Fluorescent Azide, Pink). Signal measured in FL-1 channel clearly shows the Palmitic Acid Labeling of protein.

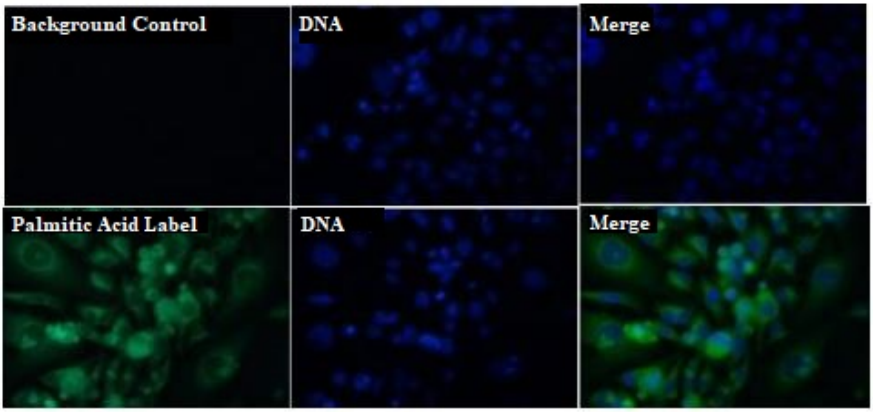


Figure 2: Metabolic labeling of palmitic acid on proliferating HeLa cells. 1 x 10^5 cells were incubated overnight with fresh aliquots of media containing Palmitic Acid Label. Upper panel corresponds to the Azide only Background fluorescence. The lower panel shows green fluorescence corresponds to the Palmitic Acid Label labeling. Nuclear staining in both panels confirms that green signal is a result of Palmitic Acid Label incorporation.

12. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

13. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)