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ab273283

Stearoylated Protein Assay Kit (Green)

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Stearoylated Protein Assay Kit, a highly specific, simple and robust method for labeling and detection of Stearoylated 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.

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1. Overview

Stearoylated Protein Assay Kit (Green) (ab273283) a highly specific, simple and robust method for labeling and detection of Stearoylated proteins. The kit uses Alkynyl Stearic Acid that is fed directly into the cells and gets incorporated into proteins during or post translation. This 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 Stearoylated proteins, or detection by FACS for quantitative studies. Labeled Stearoylated Proteins can be directly detected in 1D or 2D gels using the appropriate excitation sources or enriched by immunoprecipitation with biotin-azide or antibodies prior to proteomic analysis.

2. Protocol Summary

Culture cells of choice.



If necessary, immobilize suspension cells for microscopy.



Label the control and experimental cells. Prepare Negative Control (no Stearoylated Protein Label, no Reaction Mix) and Background Control (Reaction Mix only) cells.



Fix and permeabilize cells.



Prepare and add Reaction Mix to appropriate wells.



DNA staining.

Incubate cells for 20 mins at RT/4°C protected from light.



Wash cells.



Analyze cells by flow cytometry or fluorescence microscopy.

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 Reagent Preparation 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
1000X Stearic Acid Label/Stearoylated Protein Label (1000X)	10 µL	-20°C
100X Copper Reagent/Copper Reagent (100X)	100 µL	-20°C
100X Fluorescent Azide I/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 488 nm wavelength (FL-1)
- Fluorescence microscope capable of excitation and emission at 440/490 nm and 540/580 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 stock 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 stock 1:10 in sterile water, mix well. Store at 4°C.

9.3 Fixative Solution:

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

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

10. Assay Protocol

- 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 μ L.
- 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 Stearic Acid Label/Stearoylated Protein Label concentrations to find the best condition for your experimental design.
- Avoid stressing the cells by washes or temperature changes prior to incubation with Stearic Acid Label/Stearoylated Protein Label.
- All steps should be carried out at room temperature (RT) unless otherwise specified. Equilibrate all buffers to RT prior to the experiment.

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

- 10.1 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

- 10.2 Add 100 μ l of 0.1% gelatin solution directly into the wells.
- 10.3 Tilt the plate to cover the entire well surface and place it in a tissue culture hood for 1 hour.
- 10.4 Gently remove the gelatin solution and seed your cells. Allow the cells to recover overnight before the treatment.
- 10.5 Next day, treat the cells with appropriate effectors according to your protocol.
- 10.6 Do not add treatment to the positive and negative control cells. Negative Control Cells: Unstained cells, cells not exposed to Stearic Acid Label/Stearoylated Protein Label or Fluorescent Azide I/Fluorescent Azide. Background Control Cells: Cells are not exposed to the Stearic Acid Label/Stearoylated Protein Label, only to the Fluorescent Azide I/Fluorescent Azide Reaction. Positive Control Cells: Cells are incubated with 1X

Stearic Acid Label/Stearoylated Protein Label and Fluorescent Azide I/Fluorescent Azide Reaction.

- 10.7** The next day, for adherent cells, remove the media directly. For suspension cells: centrifuge cells at $500 \times g$ for 5 mins and discard the supernatant. Then replace it with fresh aliquots containing 1000X Stearic Acid Label/Stearoylated Protein 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 Stearic Acid Label/Stearoylated Protein Label to the Negative Control Cells.

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

Δ Note: Do not remove the drug containing media while incubating with 1X Stearic Acid Label/Stearoylated Protein Label to avoid potential reversibility of drug action on label incorporation.

- 10.9** To terminate the experiment, for adherent cells, remove the media and rinse the cells once with 100 μ L of 1X PBS, discard the supernatant. For suspension cells, centrifuge the cells at $500 \times g$ for 5 mins to deposit the cells onto the surface. Tilt the plate and gently remove the media with a pipette tip.

- 10.10** Make note of the place that is used and perform subsequent aspirations from the same place. Pellet the suspension cells at $500 \times g$ for 5 mins throughout the entire protocol.

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

Fixation and Permeabilization:

For adherent cells:

- 10.11** Add 100 μ L of Fixative Solution to each well and incubate the cells for 15 mins at RT, protected from light.
- 10.12** Remove the fixative and wash the cells once with 100 μ L of 1X Wash Buffer IV/Wash Buffer, remove the wash.

- 10.13** Add 100 μL of 1X Permeabilization Buffer and incubate the cells for 10 mins at RT.
- 10.14** Remove the supernatant and replace it with a 20 μL of 1X Permeabilization Buffer.

For suspension cells:

- 10.15** Re-suspend the cells in 100 μL of Fixative Solution and incubate for 15 mins at RT protected from light. Centrifuge the cells at 500 $\times g$ for 5 mins, remove the fixative and wash the cells once with 100 μL of 1X Wash Buffer IV/Wash Buffer.
- 10.16** Centrifuge the cells at 500 $\times g$ for 5 mins, discard the supernatant and re-suspend the cells in 100 μL of 1X Permeabilization Buffer. Incubate the cells for 10 mins at RT.
- 10.17** Centrifuge the cells at 500 $\times g$ for 5 mins.
- 10.18** Remove the supernatant and replace it with a 20 μL of Permeabilization Buffer.
- 10.19** Proceed to Stearoylated Protein reaction and total DNA staining.

Stearoylated Protein reaction and total DNA staining:

Reaction Cocktail

- 10.20** Prepare 1X Fluorescent Azide I/Fluorescent Azide Reaction Cocktail according to the table below.
- 10.21** Volumes should be multiplied by number of Samples and reagents added in the exact order.
- 10.22** Use the Fluorescent Azide I/Fluorescent Azide Reaction Cocktail within 15 mins of preparation.
- 10.23** Cells should be protected from light during and following the reaction and DNA staining.

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

Stearoylated Protein Reaction:

- 10.24** For Negative Control Cells, add 100 μ L of 1X PBS.
- 10.25** For Background Control Cells, Positive Control Cells and Experimental Cells: Add 100 μ L of 1X Reaction Cocktail to each Sample and incubate the cells for 30 mins at RT protected from light.
- 10.26** Remove the Reaction Cocktail and wash cells three times in 100 μ L of 1X Wash Buffer IV/Wash Buffer.
- 10.27** Remove the 1X wash and suspend the cells in 100 μ L of 1X PBS. Proceed to DNA staining.
- 10.28** If no DNA staining is desired, proceed to Microscopic or FACS analysis.

DNA staining:

- 10.29** Prepare 1X dilution of DAPI/Total DNA Stain and add 100 μ L per well.
- 10.30** Incubate the cells for 20 mins at RT or refrigerate at 4°C protected from light.
- 10.31** Remove the DNA stain solution. Wash the cells once with 100 μ L PBS

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

Flow Cytometry:

- 10.32** Analyze samples by flow cytometry (Ex = 488 nm).
- 10.33** Harvest cells by preferred method and wash with 0.5 mL of ice-cold PBS. Resuspend pellets with 100 μ L of ice-cold PBS and analyze Samples for red fluorescence generated by Stearic Acid Label/Stearoylated Protein Label addition during the labeling reaction.

Fluorescence Microscope Imaging:

- 10.34** Fluorescence microscope capable of excitation and emission at 440/490 nm (Ex=440/Em=490 nm) and UV filter.
- 10.35** Harvest cells by preferred method and wash with 0.5 mL of ice-cold PBS.
- 10.36** Resuspend pellets with 100 μ L of ice-cold PBS and analyze Samples for red fluorescence generated by the Stearic Acid

Label/Stearoylated Protein Label addition during the labeling reaction.

11. Typical Data

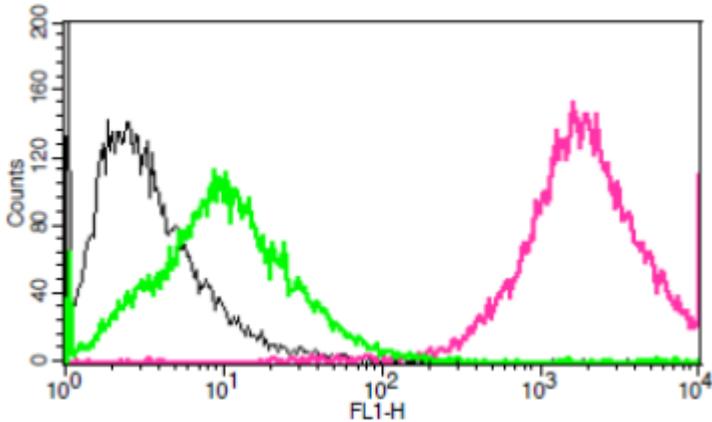


Figure1: Metabolic labeling of Stearoylated Protein on proliferating Jurkat cells. 1×10^6 cells were incubated overnight with fresh aliquots of media containing Stearic Acid Label/Stearoylated Protein Label: Flow cytometry analysis of Negative Control (Black), Background (Fluorescent Azide I/Fluorescent Azide only, Green), Positive Control (Stearic Acid Label/Stearoylated Protein Label and Fluorescent Azide I/Fluorescent Azide, Red). Signal measured in FL-1 channel clearly shows the Stearic Acid Label/Stearoylated Protein Labeling of Protein.

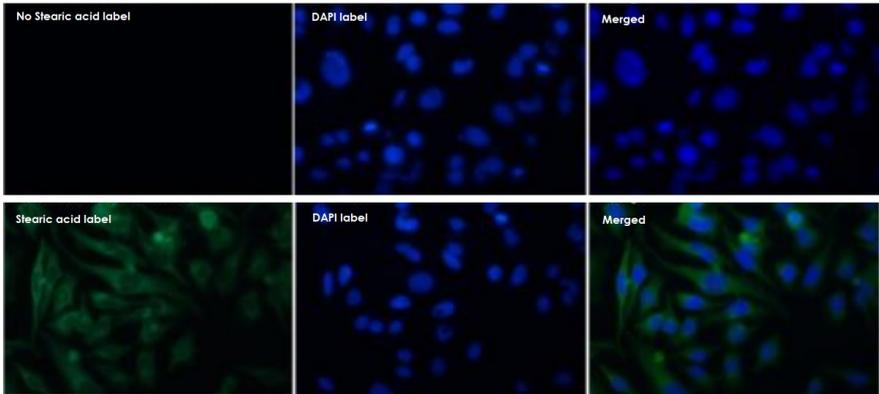


Figure 2: Metabolic labeling of Stearoylated Protein on proliferating HeLa cells. 1×10^5 cells incubated overnight with fresh aliquots of media containing Stearic Acid Label/Stearoylated Protein Label: Upper panel corresponds to the Fluorescent Azide I/Fluorescent Azide only Background fluorescence on HeLa cells. The lower panel shows that the red fluorescence corresponds to the Stearic Acid Label/Stearoylated Protein Label labeling reaction. Nuclear staining in both panels confirms that green signal is a result of Stearic Acid Label/Stearoylated Protein Label incorporation.

12.FAQ / Troubleshooting

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

13. Notes

Technical Support

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