Version 2 Last updated 10 July 2020

ab241025 Phospholipid Synthesis Assay Kit

View Phospholipid Synthesis Assay Kit datasheet: www.abcam.com/ab241025

For the Detection and quantification of biosynthesis, subcellular localization and turnover of phospholipids in suspension or adherent cells.

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

Copyright © 2018 Abcam. All rights reserved

Table of Contents

Copyright © 2018 Abcam. All rights reserved

1. Overview

The Phospholipid Synthesis Assay Kit (ab241025) offers a simple and robust method to label and visualize newly synthesized phospholipids *in vivo*. Based on the metabolic incorporation of the choline analogs directly into their structure, modified phospholipid molecules can be detected with high

sensitivity and spatial resolution by click chemistry with azide-containing dyes.

This kit enables analyses of global biosynthesis, subcellular localization and turnover of Cho-containing phospholipids in cells. Cells show strong incorporation of Cho analogs into all classes of phospholipids that can be assayed by fluorescence microscopy, or quantified by FACS.

2. Protocol Summary

Prepare all samples and controls as instructed and incubate experimental cells with your chosen effectors.

Label the control and experimental cells by incubation with 1X diluted phospholipid label. Incubate for 24 hours at 37°C.

Terminate the experiment by rinsing with PBS. For immobilized suspension cells centrifuge at 500 X g for 5 minutes.

Fix and permeabilize the cells using 100 µL Fixative solution and 20 µL permeabilization Buffer.

Prepare the Reaction cocktail and add 100 µL to each sample and incubate for 30 minutes in the dark at RT.

Analyze the samples by either fluorescence microscopy or FACS analysis.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
Wash Buffer	25 mL	-20°C
Fixative Solution	10 mL	-20°C
Permeabilization Buffer	25 mL	-20°C
Phospholipid Label (1000X)	10 µL	-20°C
Copper Reagent (100X)	100 µL	-20°C
Fluorescent Azide (100X)	100 µL	-20°C
Reducing Agent (20X)	500 µL	-20°C
Total DNA Stain (1000X)	10 µL	-20°C

5. 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; flow cytometry vessels
- Phosphate Buffered Saline (PBS, pH 7.4)
- Sterile 0.1% Gelatin Solution (optional, only required for adhering suspension cells to the surface)
- Flow cytometer equipped with laser capable of excitation at 488 and 530/590 nm emission filters respectively
- Fluorescence microscope capable of excitation and emission at 440/490 and 540/580 nm respectively.

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 Wash Buffer:

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

6.2 Fixative Solution:

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

6.3 Permeabilization Buffer (10X):

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

6.4 All other components:

Ready to use as supplied. Store at -20°C protected from light. While in use, keep on ice and minimize light exposure.

7. Sample Preparation

7.1 Suspension and adherent cells:

- 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:** add 100 µL of 0.1% gelatin solution into each well of a tissue culture plate, tilt the plate to cover the entire well surface and 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.
- Next day, remove the media and treat the cells with appropriate effectors according to your protocol; do not add treatment to the positive and negative control cells.
- **Negative control -** cells not exposed to the Phospholipid Label or treatment).
- **Positive control -** (cells incubated with 1X Phospholipid Label only.

8. Assay Procedure

8.1 Labeling control and experimental cells:

8.1.1 Dilute Phospholipid Label (1000X) to 1X final concentration with culture medium and add into the experimental and positive control cells respectively.

8.1.2 Do not add the Phospholipid Label into the negative control cells. Do not remove the drug containing media during incubation with 1X Phospholipid Label to avoid potential reversibility of drug action on label incorporation.

8.1.3 Incubate the cells for an additional 24 hours at 37°C.

8.1.4 Terminate the experiment by removal of the culture medium and rinsing cells once with 100 µL of PBS, discard the supernatant.

8.1.5 For immobilized suspension cells: Centrifuge the plate at 500 x g (or the lowest centrifuge setting) for 5 minutes to gently deposit the cells onto the surface. Tilt the plate and gently remove the media with a pipette tip. It is important to avoid excessive centrifugation speeds, which can damage the cells. Make note of the place that is used, and perform subsequent aspirations from the same place.

8.1.6 Proceed to the Fixation and Permeabilization.

8.2 Fixation and permeabilization:

For adherent cells:

8.2.1 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 twice with 100 µL of 1X Wash Buffer, remove the wash.

8.2.2 Add 100 µL of 1X Permeabilization Buffer and incubate the cells for 10 min at RT. Remove the Permeabilization Buffer and replace it with a 20 µL of fresh aliquot.

For suspension cells:

8.2.3 Re-suspend the cells in 100 µL of Fixative Solution and incubate for 15 min at RT protected from light. Remove the fixative and wash the cells twice with 0.5 ml of 1X Wash Buffer.

8.2.4 Discard the supernatant and re-suspend the cells in 100 µL of 1X Permeabilization Buffer. Incubate the cells for 10 min at RT. Remove the Permeabilization Buffer and replace it with a 20 µL of fresh aliquot. Proceed to reaction and total DNA staining.

Reaction Mix:

8.3 Prepare enough Reaction Mix for the number of assays to be performed. For each well, prepare 100 µL Reaction Mix containing:

	Reaction Mix
PBS	93 µL
Cooper Reagent	1 µL
Fluorecent Azide (100X)	1 µL
Reducing Agent	5 µL

8.4 Add 100 µL of Reaction Mix to each well containing the test sample and incubate the cells for 30 minutes in the dark at RT.

8.5 Remove the reaction cocktail and wash cells three times in 100 µL of Wash Buffer. Remove the wash and suspend the cells in 100 µL of PBS.

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

DNA staining:

8.7 Prepare 1X dilution of Total DNA Stain and add 100 µL per well. Incubate the cells for 20 minutes at room temperature, or refrigerate at 4 °C protected from light.

8.8 Remove the stain solution; wash the cells once with 100 µL of PBS

ΔNote: cells are compatible with all methods of slide preparation including wet mount or prepared mounting media.

9. Fluorescence Microscope and FACS analysis:

9.1 Analyze samples for red fluorescence generated by labeled phospholipid and green by total DNA respectively.

9.2 **FACS Analysis:** Harvest the cells by preferred method and wash with 0.5 ml of ice-cold PBS. Re-suspend the pellets in 100 µL of ice-cold PBS.

9.3 Transfer the cell suspension into flow cytometry vessels. Analyze samples in FL-2 channel for signal generated by phospholipid during click reaction.

ΔNote: Trypsin can be used to collect the adherent cells prior to FACS analysis.

10. Typical Data

Typical data provided **for demonstration purposes only**.

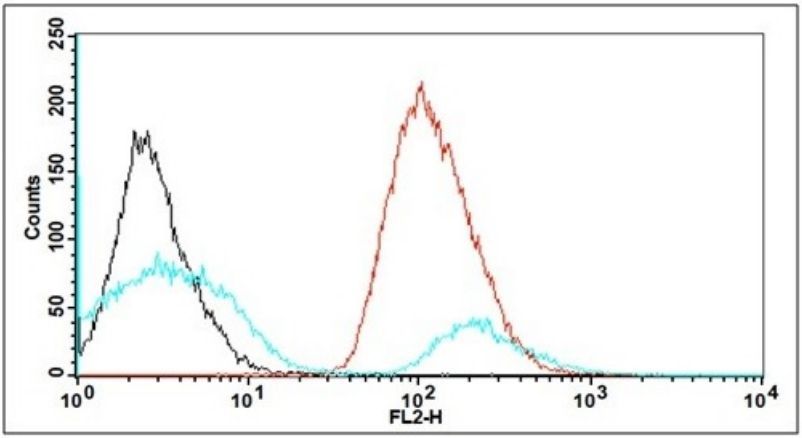

Figure 1. Analysis of metabolic labeling of phospholipids in proliferating cells. Jurkat cells (1X10^6 cells/ml) were pre-treated with vehicle (black line) or cultured in presence of 1X Phospholipid Label (red line) for 24 hours at 37°C prior to 1 hour incubation with Phospholipase D (blue line). Modified phospholipid molecules were detected according to the kit protocol and red fluorescence was analyzed by FACS in FL-2 channel. Decrease in signal caused by hydrolysis of Cho-containing head groups via Phospholipase D activity confirms that red fluorescence is the result of Phospholipid Label incorporation.

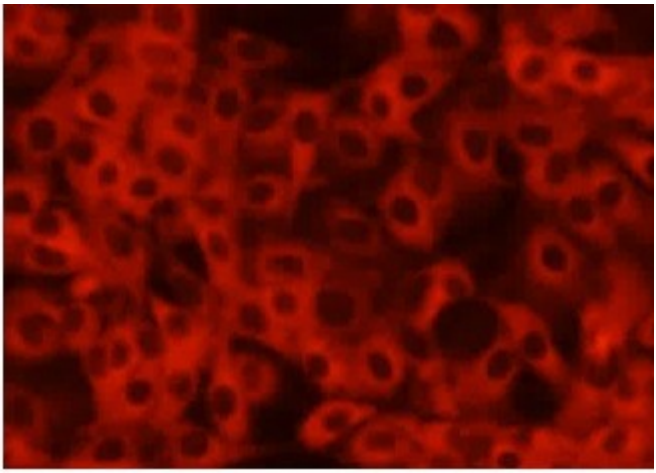

Figure 2. BALB/3T3 cells (10^5 cells/ ml) cultured in presence of Phospholipid Label for 24 hours at 37°C and processed according to kit protocol. Choline-containing phospholipids were detected by Fluorescence Microscope. All experiments were followed according to the kit protocol.

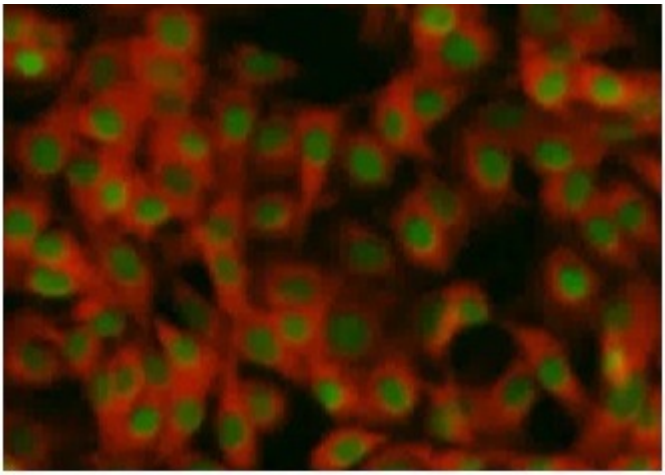

Figure 3. Total DNA staining confirms that red fluorescence is the result of Phospholipid Label incorporation.

11. Notes

Technical Support

Copyright © 2018 Abcam. All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.

Austria
wissenschaftlicherdienst@abcam.com | 019-288-259
France
supportscientifique@abcam.com | 01.46.94.62.96
Germany
wissenschaftlicherdienst@abcam.com | 030-896-779-154
Spain
soportecientifico@abcam.com | 91-114-65-60

Switzerland
technical@abcam.com
Deutsch: 043-501-64-24 | Français: 061-500-05-30
UK, EU and ROW
technical@abcam.com | +44(0)1223-696000

Canada
ca.technical@abcam.com | 877-749-8807
US and Latin America
us.technical@abcam.com | 888-772-2226

Asia Pacific
hk.technical@abcam.com | (852) 2603-6823
China
cn.technical@abcam.com | 400 921 0189 / +86 21 2070 0500
Japan
technical@abcam.co.jp | +81-(0)3-6231-0940
Singapore
sg.technical@abcam.com | 800 188-5244

Australia
au.technical@abcam.com | +61-(0)3-8652-1450
New Zealand
nz.technical@abc.com | +64-(0)9-909-7829

Copyright © 2018 Abcam. All rights reserved