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ab273294 Total Phospholipid Assay Kit (Cell-Based)

View Kit datasheet: https://www.abcam.com/ab273294
(use https://www.abcam.cn/ab273294 for china, or
https://www.abcam.co.jp/ab273294 for Japan)

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

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

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1. Overview

Total Phospholipid Assay Kit (Cell-Based) (ab273294) 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 (Ex/Em= 494/521 nm). This kit enables quantitative analyses of global biosynthesis/turnover of Cho-containing phospholipids in cells. Cells show strong incorporation of Cho analogs into all classes of phospholipids that can be assayed by microplate reader and fluorescence microscope. The kit provides sufficient materials for 100 assays.

2. Protocol Summary

Prepare cells as directed

⇩

Prepare all reagents as directed

⇩

Treat cells overnight, then remove media

⇩

Add Phospholipid Label to Experimental and Positive Control cells, and incubate for 24 hours at 37°C

⇩

Fix, wash and permeabilize cells

⇩

Add Reaction cocktail (100 µL) and incubate for 30 minutes at RT protected from light

⇩

Either/ Analyze immediately in the plate reader at Ex/Em= 494/521 nm in end point mode

Or/ Image with fluorescence microscope directly in the plate following DNA staining.

⇩

Prepare Azide Fluorescence Curve from same cell suspension in parallel to the experimental treatment for each cell line and condition

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.

Aliquot components in working volumes before storing at the recommended temperature.

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)	Storage temperature (after prep)
Wash Buffer (10X)	25 mL	-20°C	+4°C
Fixative Solution	10 mL	-20°C	+4°C
Permeabilization Buffer (10X)	25 mL	-20°C	+4°C
Phospholipid Label (1000X)	10 µL	-20°C	-20°C
Copper Reagent (100X)	100 µL	-20°C	-20°C
Fluorescent Azide (100X)	100 µL	-20°C	-20°C
Reducing Agent (20X)	500 µL	-20°C	-20°C
Total DNA Stain (1000X)	20 µL	-20°C	-20°C

7. Materials Required, Not Supplied

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

- Multi-well spectrophotometer capable of measuring Ex/Em= 494/521 nm
- Fluorescence microscope (optional) capable of measuring Ex/Em= 494/521 nm spectra
- Tissue culture vessels and appropriate culturing media
- A 6-, 12-, 24-, or 96-well clear plates should be used only for cell culturing. The measurement of fluorescence should be performed in black opaque plates. Alternatively, sterile opaque plates can be used for both, culturing and measurements
- Phosphate Buffered Saline (PBS, pH 7.4)

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.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 **Wash Buffer (10X):**
Thaw at 37°C to dissolve completely. Dilute the 10X stock 1:10 in sterile water, mix well. Store at 4 °C.

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

9.3 **Permeabilization Buffer (10X):**
Thaw at 37°C to dissolve completely. Dilute the 10X stock 1:10 in sterile water, mix well. Store at 4 °C.

9.4 **Phospholipid Label (1000X):**
Ready to use. Store at -20°C protected from light. While in use, keep on ice and minimize light exposure.

9.5 **Copper Reagent (100X):**
Ready to use. Store at -20°C protected from light. While in use, keep on ice and minimize light exposure.

9.6 **Fluorescent Azide (100X):**
Ready to use. Store at -20°C protected from light. While in use, keep on ice and minimize light exposure.

9.7 **Reducing Agent (20X):**
Ready to use. Store at -20°C protected from light. While in use, keep on ice and minimize light exposure.

9.8 **Total DNA Stain (1000X):**
Ready to use. Store at -20°C protected from light. While in use, keep on ice and minimize light exposure.

10. Sample Preparation

Δ Note: This assay was developed with Jurkat (suspension) and HeLa (adherent) cells and can be modified for any cell line. The following protocol has been optimized for a 96-well opaque plate at $1X10^6$ cells per well, using fewer cells per well or clear plate will result in decreased signal. We suggest testing growth conditions, cell number per well and several concentrations of the Phospholipid Label, to find the best experimental design for your cell type. The assay volume is 100 µL; adjust volumes accordingly for other plate formats. Avoid stressing the cells by washes or temperature changes prior to incubation with Phospholipid Label. All steps should be carried out at room temperature unless otherwise specified; equilibrate all buffers to room temperature prior to the experiment.

11. Assay Procedure

Thaw all reagents thoroughly and mix gently.

Δ Note: This assay was developed with Jurkat (suspension) and HeLa (adherent) cells and can be modified for any cell line. The following protocol has been optimized for a 96-well opaque plate at $1X10^6$ cells per well, using fewer cells per well or clear plate will result in decreased signal. We suggest testing growth conditions, cell number per well and several concentrations of the Phospholipid Label, to find the best experimental design for your cell type. The assay volume is 100 µL; adjust volumes accordingly for other plate formats. Avoid stressing the cells by washes or temperature changes prior to incubation with Phospholipid Label. All steps should be carried out at room temperature unless otherwise specified; equilibrate all buffers to room temperature prior to the experiment.

11.1 Labeling of control and experimental cells:

11.1.1 Plate suspension or adherent cells at a desired density and allow for overnight recovery before treatment. Ensure that adherent cells are sub-confluent. Include appropriate controls and account for cell loss during the processing. **Negative control** (cells not exposed to the Phospholipid Label or treatment), **Background control** (cells treated with cocktail only), **Positive control** (cells incubated with 1X Phospholipid Label only).

11.1.2 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.

11.1.3 For **suspension cells**: Centrifuge the plate at 500 x *g* (or the lowest centrifuge setting) for 5 minutes at RT to pellet the cells. Tilt the plate and gently remove the media with a pipette tip. Avoid excessive centrifugation speeds, which can damage the cells. Use these centrifugation settings throughout the entire protocol.

11.1.4 Dilute Phospholipid Label (1000X) to 1X final concentration with culture medium and add into the Experimental and Positive control cells respectively. Incubate the cells for additional 24 hours, or time required by your experimental protocol in a 37°C incubator. 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. Harvest the suspension cells by centrifugation. Optionally, detach **adherent cells** (e.g. trypsinize and quench with media), and harvest by centrifugation. Wash the cells once with 100 µL of PBS, discard the supernatant.

11.2 **Fixation and Permeabilization:**

11.2.1 For **adherent and suspension cells**: Add 100 µL of Fixative Solution to each well and incubate the cells for 15 minutes at room temperature protected from light.

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

11.2.3 Add 100 µL of 1X Permeabilization Buffer and incubate the cells for 10 minutes at room temperature.

11.2.4 Remove the Permeabilization Buffer and replace it with a 20 µL of a fresh aliquot.

11.3 **Reaction cocktail:**

11.3.1 Prepare 1X reaction cocktail according to the table below. Volumes should be multiplied by number of samples and reagents and added in the exact order. Use the reaction cocktail within 15 minutes of preparation. Cells should be protected from light during, and following the reaction and DNA staining.

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

11.3.2 Add 100 µL of 1X Reaction cocktail to each sample and incubate the cells for 30 minutes at room temperature protected from light.

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

11.3.4 Suspend the cells in 100 µL of Wash Buffer.

11.4 **Detection:**

11.4.1 Cells must be analyzed immediately in the plate reader at Ex/Em= 494/521 nm in end point mode to determine change in fluorescence of compounds and controls after background subtraction, or imaged with fluorescence microscope directly in the plate following DNA staining.

11.4.2 **DNA staining**: Prepare 2X 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. Remove the DNA stain and re-suspend the cells in 100 µL of PBS prior to imaging. Analyze samples for green fluorescence generated by *de novo* synthesized phospholipid and for blue fluorescence by nuclear DNA.

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

11.5 **Azide Fluorescence Curve:**

11.5.1 To increase the accuracy of your data, an azide fluorescence curve should be prepared from the same cell suspension in parallel to the experimental treatment for each cell line and condition.

11.5.2 In a 96-well opaque plate, prepare a series of dilutions of your cell suspension starting with the same volume and number of cells as in the experimental wells.

11.5.3 Dilute the cells by a factor of 2. Optional: To minimize the error, aliquot at least 3 wells per dilution.

11.5.4 Measure fluorescence and calculate average for each dilution, subtract the background value.

11.5.5 Plot the Azide Fluorescence Curve to obtain fluorescence per cell number and the detection limit for your assay.

11.5.6 Also, a standard curve of Fluorescent Azide concentration per well can be prepared in the same manner to obtain the least detectable amount of azide for your experiment.

12. Typical Data

Typical fluorescence curve – data provided **for demonstration purposes only**. A new curve must be generated for each assay performed. Data will depend on the cell type and tested compound.

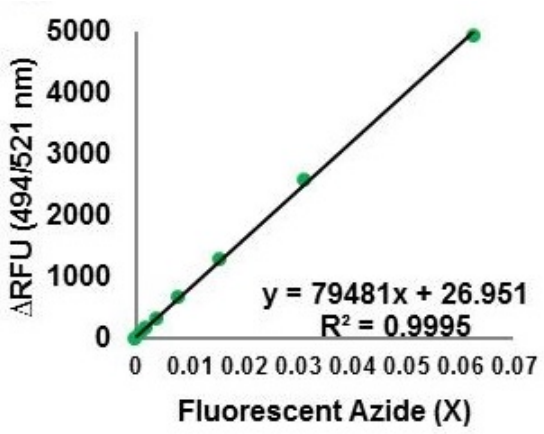

Figure 1. Azide Fluorescence Curve in 0-0.1 X range.

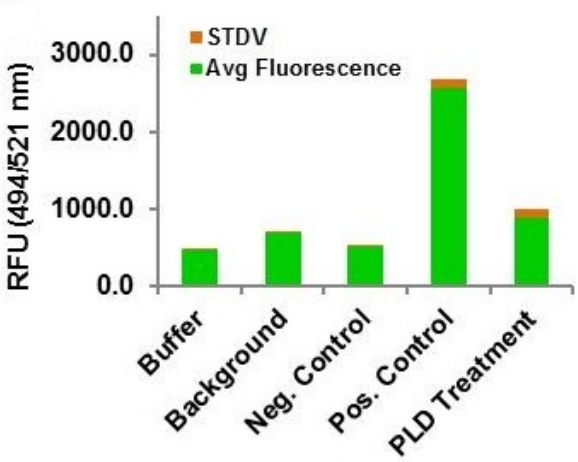

Figure 2. Analysis of metabolic labeling of phospholipids in proliferating cells. Jurkat cells (1 x 10^6 cells/well) were pre-treated with vehicle or cultured in presence of 1X Phospholipid Label for 24 hours at 37°C prior to 1 hour treatment with Phospholipase D and then processed for detection according to the kit protocol. Plate reader analyses of controls and PLD treatment; Avg fluorescence +/- standard deviation plotted for 3 replicates per condition.

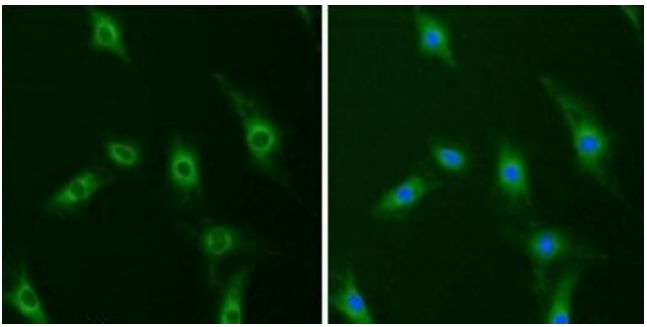

Figure 3. Analysis of metabolic labeling of phospholipids in proliferating cells. BALB/3T3 cells seeded at 10^5 cells/ ml were pre-treated with vehicle or cultured in presence of 1X Phospholipid Label for 24 hours at 37°C prior to 1 hour treatment with Phospholipase D and then processed for detection of according to the kit protocol. BALB/3T3 cells: left panel- green fluorescence of *de novo* synthesized phospholipids; right panel-nuclear staining and phospholipid images merged.

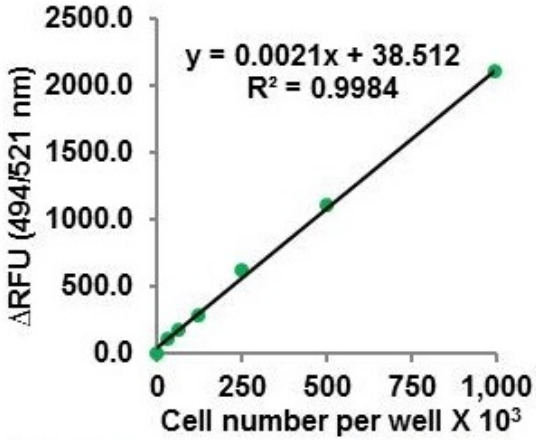

Figure 4. Analysis of metabolic labeling of phospholipids in proliferating cells. Jurkat cells (1X10^6 cells/well) were pre-treated with vehicle or cultured in presence of 1X Phospholipid Label for 24 hours at 37°C prior to 1 hour treatment with Phospholipase D and then processed for detection of according to the kit protocol. Fluorescence Azide Curve of Jurkat cells prepared for this assay. Detection limit corresponds to about 31,250 of Jurkat cells per well. Your results may not be identical to these. A new curve must be obtained for each experiment and the cell line.

13. FAQ / Troubleshooting

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

14. 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)