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ab241036
Cardiolipin Assay Kit

For the measurement of Cardiolipin content in cell lysates and isolated mitochondria.

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

Table of Contents

1. Overview

The Cardiolipin Assay Kit (ab241036) is a fluorometric assay that makes use of a proprietary probe that fluoresces on association with cardiolipin but not with any other lipids such as phosphatidylcholine and sphingomyelin, making it highly specific.

Fluorescence can be recorded at Ex/Em 340/480 nm. The kit includes purified cardiolipin as standard and can detect as low as 0.2 nmol of cardiolipin.

2. Protocol Summary

Prepare all samples, controls and standards as instructed.

Prepare the standard curve using the 5 mM cardiolipin standard. Dilute to 250 µM using Assay Buffer 16.

Create the Reaction Mix, add 50 µL to each well.

Incubate the plate at room temperature for 5-10 minutes. Read the fluorescence at EX/EM 340/480 nm.

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.
- Briefly centrifuge small vials prior to opening.

Item	Quantity	Storage condition
Assay Buffer 16	25 mL	-20°C
CL Probe	1 vial	-20°C
Cardiolipin Standard	1 vial	-20°C

PLEASE NOTE: Assay Buffer 16 was previously labelled as Assay Buffer XVI and CL Assay Buffer, and Cardiolipin Standard as Cardiolipin (5 µmM). The composition has not changed.

5. Materials Required, Not Supplied

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

- 96-well white plate with flat bottom
- Multi-well spectrophotometer
- Deionized water
- Ethanol

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 Assay Buffer 16:

Bring to room temperature before use.

6.2 CL Probe:

Reconstitute in 220 µL deionized water and aliquot. Reconstituted probe is stable for 3 months, when stored at -20°C. Do not reconstitute in Assay Buffer 16.

6.3 Cardiolipin:

Reconstitute in 25 µL Ethanol. Store at -20°C. Do not warm to 37°C or leave uncapped.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

7.1 Dilute the 5 mM Cardiolipin Standard 1:20 in Assay Buffer 16 to obtain 250 µM Cardiolipin standard.

7.2 Add 0, 2, 4, 6, 8, 10 and 12 µL of the 250 µM Cardiolipin standard to wells of the 96 well white plate.

7.3 Bring the total volume of each well to 50 µL with Assay Buffer 16 to generate 0, 0.5, 1, 1.5, 2, 2.5 and 3 nmol/well of cardiolipin.

Standard #	250 µM Cardiolipin standard (µL)	Assay Buffer 16 (µL)	Cardiolipin concentration Per well (nmol)
1	12	38	3
2	10	40	2.5
3	8	42	2
4	6	44	1.5
5	4	46	1
6	2	48	0.5
7	0	50	0

ΔNote: Place the 5 mM cardiolipin standard on ice while making the 250 µM solution and do not leave it uncapped.

ΔNote: Immediately store the remaining 5 mM cardiolipin standard at -20 °C. When stored at -20 °C, it is stable for several months.

8. Sample Preparation

- Cell lysates or isolated mitochondria may be used for quantification of cardiolipin.

8.1 Cell lysates:

- Suspend cells in Assay Buffer 16 and carry out detergent free lysis of cells (using sonicator, freeze thawing or another preferred method of lysis).
- Centrifuge at 10,000 x g for 10 minutes at 4°C and transfer the supernatant to a fresh tube.

8.2 Isolated Mitochondria:

- Use preferred procedure to isolate mitochondria from cultured cells or tissue.
- Determine protein concentration of cell lysate or isolated mitochondrial samples using preferred method.
- Add 2 to 20 µL of samples into wells of a 96-well white plate (15 - 90 µg protein for cell lysates, and 10 - 40 µg protein for isolated mitochondria).
- For each sample prepare two wells; "Sample background control" and "Sample". Bring the volume in "Sample" wells to 50 µL and in "Sample background control" to 100 µL using CL buffer.

ΔNote: Different dilutions of the mitochondrial sample should be tested to make sure that cardiolipin concentration falls in the linear range of the assay. Samples should be diluted using CL Buffer.

9. Assay Procedure

9.1 Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µL Probe Mix containing the following components. Mix well before use:

	Probe Mix
Assay Buffer 16	48 µL
CL Probe	2 µL

9.2 Add 50 µL of the Probe Mix to each well containing the Cardiolipin Standard(s), Sample(s), Mix well.

9.3 Do not add this mix to Sample background control wells.

9.4 Incubate at room temperature for 5-10 minutes.

9.5 **Measurement:** Record the fluorescence at Ex/Em 340/480 nm.

10. Data Analysis

10.1 Subtract the 0 Cardiolipin Standard reading from all Standard curve readings. Plot the background-subtracted Cardiolipin Standard Curve and calculate the slope.

10.2 If sample background control is significant, then subtract sample background control reading from sample readings.

10.3 Apply the corrected ΔRFU value to the Cardiolipin Standard Curve to get B nmol cardiolipin in the sample well.

$$\text{Sample Cardiolipin Concentration} = (B/V) \times D \text{ nmol/mL}$$

Where:

B is the amount of Cardiolipin in the sample well from Standard Curve (nmol)

V is the sample volume added into the reaction well (mL)

D is the sample dilution factor

Cardiolipin molecular weight: 1501 g/mol

Cardiolipin concentrations can also be expressed as nmol Cardiolipin per mg protein.

11. Typical Data

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

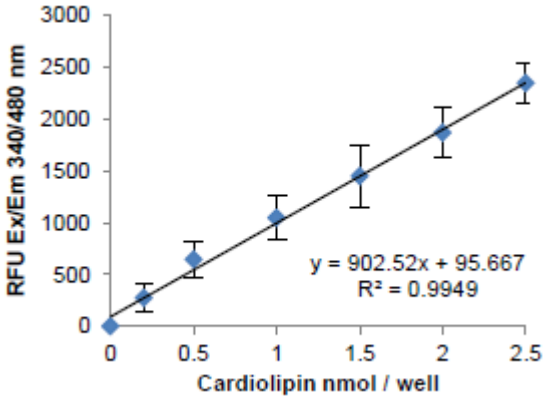

Figure 1. Cardiolipin Standard Curve after subtraction of background.

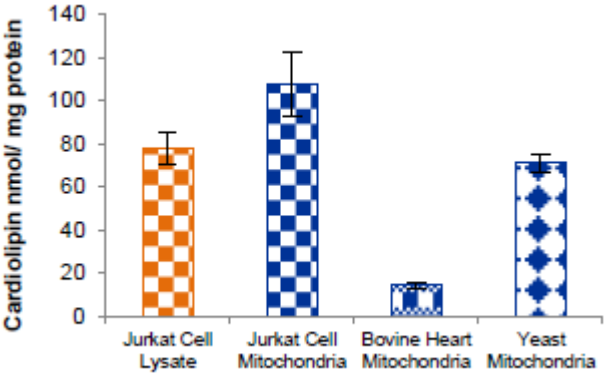

Figure 2. Cardiolipin content per mg protein in Jurkat cell lysate, Jurkat cell mitochondria, Bovine heart mitochondria and Yeast Mitochondria (S. cerevisiae).

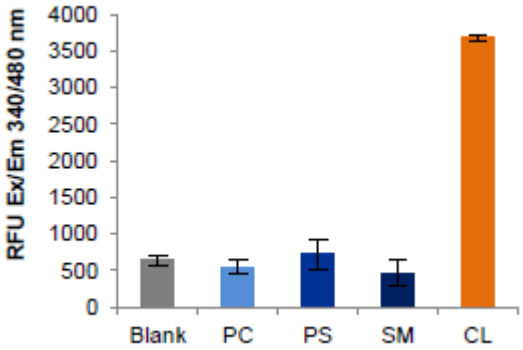

Figure 3: Signal from phosphatidylcholine (PC), phosphatidylserine (PS), sphingomyelin (SM) and cardiolipin (CL) demonstrate the specificity of the Cardiolipin Probe. Known amount of each lipid was added (5 nmol/well). Assay was performed using kit protocol.

12. 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)