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Product Information

Cardiolipin Assay Kit

Catalog Number **MAK362**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Cardiolipin (CL) is a very important phospholipid present in the inner mitochondrial membrane and constitutes about 20% of its total lipid composition. It has a dimeric structure comprised of two phosphatidic acid residues linked by a glycerol bridge. It is essential for several mitochondrial functions such as maintaining activity of the electron transport chain complexes, and other mitochondrial processes including biogenesis, fission, fusion, and protein transport. It is involved in apoptosis where it helps alter the mitochondrial membrane structure and aids in the release of cytochrome c. Conditions such as diabetes and heart failure are linked to changes in levels of cardiolipin and it is also depleted during aging. Exposure to toxicants like cigarette smoke and organophosphates may cause an alteration in cardiolipin levels and composition, thus adversely affecting health.

The Cardiolipin Assay Kit 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 λ_{ex} = 340 nm/λ_{em} = 480 nm. The kit includes purified cardiolipin as standard and can detect as low as 0.2 nmol of cardiolipin.

The kit is suitable for the measurement of cardiolipin content in cell lysates and isolated mitochondria.

Components

The kit is sufficient for 100 fluorometric assays in 96 well plates.

CL Assay Buffer Catalog Number MAK362A	25 mL
CL Probe Catalog Number MAK362B	1 vial
Cardiolipin (5 mM) Catalog Number MAK362C	20 µL

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- White flatbottom 96 well plates
- Fluorescence multiwell plate reader
- Refrigerated microcentrifuge capable of RCF ≥10,000 × *g*
- Bicinchoninic Acid Kit for Protein Determination (Catalog Number BCA1)
- Mitochondria Isolation Kit

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at –20 °C, protected from light.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

CL Assay Buffer: Warm to room temperature prior to use.

CL Probe: Reconstitute in 220 µL of ultrapure water and aliquot. Reconstituted probe is stable for 3 months, when stored at –20 °C. Do not reconstitute in assay buffer.

Cardiolipin (5 mM): Keep on ice while in use and do not leave uncapped. Immediately place unused portion at –20 °C when not in use.

Procedure

Sample Preparation

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

Mitochondria Isolation: Isolate mitochondria using preferred method for maximum yield and result consistency.

Sample Addition

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

1. Determine the protein concentration of the cell lysate or isolated mitochondrial samples.
2. Add 2–20 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).
3. For each sample prepare two wells; "Sample background control" and "Sample".
4. Bring the volume in "Sample" wells to 50 µL and in "Sample background control" to 100 µL using CL Assay buffer.

Standard Curve Preparation

Prepare a 250 µM cardiolipin standard by diluting the 5 mM cardiolipin 20-fold with CL Assay Buffer. Prepare Cardiolipin Standards in desired wells of a white 96 well plate according to Table 1.

Table 1.
Preparation of Cardiolipin Standards

Well	250 µM Premix	CL Assay Buffer	Cardiolipin (nmol/well)
1	0 µL	50 µL	0
2	2 µL	48 µL	0.5
3	4 µL	46 µL	1
4	6 µL	44 µL	1.5
5	8 µL	42 µL	2
6	10 µL	40 µL	2.5
7	12 µL	38 µL	3

Reaction Mix

Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µL by mixing:

48 µL CL Assay Buffer
2 µL CL Probe

Add 50 µL of the reaction mix to wells of the 96 well plate containing the samples and standards. Mix well.
Note: Do Not Add this mix to "Sample background control" wells.

Measurement

Incubate plate at room temperature for 5-10 minutes. Record fluorescence at λ_{ex} = 340 nm/λ_{em} = 480 nm.

Results

1. Subtract 0 Cardiolipin reading from all readings.
2. Plot the Cardiolipin Standard Curve.
3. If sample background control is higher than 0 cardiolipin, then subtract sample background control reading from sample reading.
4. Compare corrected RFU to Standard Curve to get nmol Cardiolipin (B) in the sample well.

Cardiolipin concentration in sample (nmol/mL) =

$$(B/V) \times D$$

where:
B = amount of cardiolipin in the sample well from Standard Curve (nmol)
V = volume of sample added into the well (µL)
D = dilution factor

Cardiolipin molecular weight: 1,501 g/mol
Cardiolipin concentrations can also be expressed as nmol Cardiolipin per mg protein.

Figure 1.
Typical Cardiolipin Standard Curve after Subtraction of Background

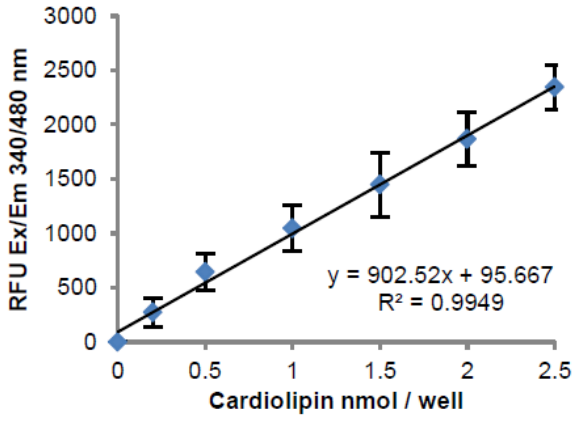
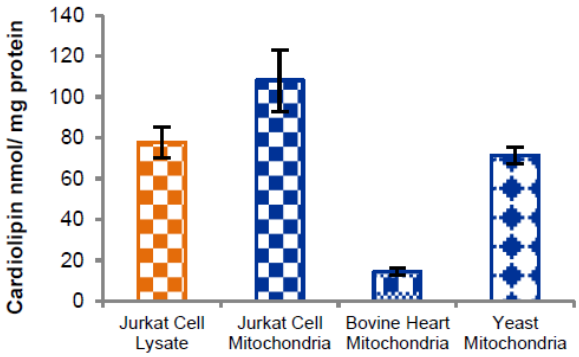
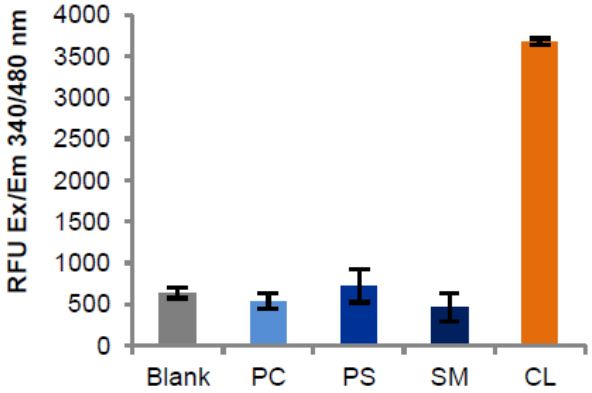


Figure 2.
Cardiolipin Content



Cardiolipin content per mg protein in Jurkat cell lysate, Jurkat cell mitochondria, bovine heart mitochondria, and yeast mitochondria (*S. cerevisiae*).

Figure 3.
Specificity of the Cardiolipin Probe



Signal from phosphatidylcholine (PC), phosphatidyl-serine (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.

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