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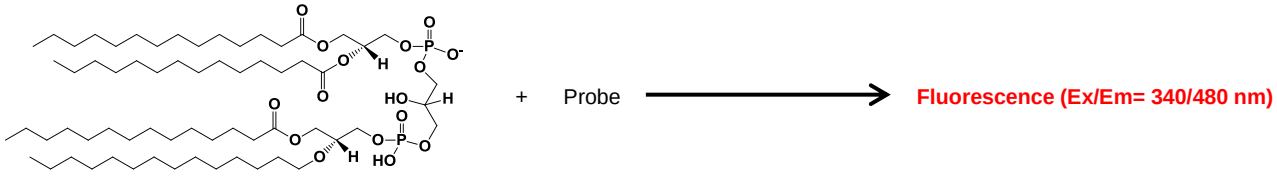
Cardiolipin Assay Kit (Fluorometric)

11/16

(Catalog # K944-100; 100 assays; Store at -20°C)

I. Introduction:

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. Condition 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. BioVision's Cardiolipin Assay 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.



II. Applications:

- Measurement of Cardiolipin content in cell lysates and isolated mitochondria

III. Sample Type:

- Cell Lysate
- Isolated Mitochondria

IV. Kit Contents:

Components	K944-100	Cap Code	Part Number
CL Assay Buffer	25 ml	WM	K944-100-1
CL Probe	1 vial	Red	K944-100-2
Cardiolipin (5 mM)	20 µl	Yellow	K944-100-3

V. User Supplied Reagents and Equipment:

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

VI. Storage Conditions and Reagent Preparation:

Upon arrival, store the kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **CL Assay Buffer:** Warm to room temperature before use.
- **CL Probe:** Reconstitute in 220 µl deionized water and aliquot. Reconstituted probe is stable for 3 months, when stored at -20ºC. *Do not reconstitute in assay buffer.*
- **Cardiolipin:** Store at -20°C. Do not warm at 37°C or leave uncapped.

VII. Cardiolipin Assay Protocol:

1. **Sample Preparation:** Cell lysates or isolated mitochondria may be used for quantification of cardiolipin**.** 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 x g for 10 minutes at 4°C and transfer the supernatant to a fresh tube. For isolated mitochondria, use preferred procedure to isolate mitochondria from cultured cells or tissue. We recommend Mitochondria Isolation Kit for Tissue & Cultured Cells (BV Cat # K288-50) and Yeast Mitochondria Isolation Kit (BV Cat # K259-50) for maximum yield and result consistency. Determine protein concentration of cell lysate or isolated mitochondrial samples using preferred method. We recommend BV# K813-2500. 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.

   Note: 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.

2. **Cardiolipin Standard Curve Generation:** Dilute the provided 5 mM cardiolipin 1:20 in CL Assay Buffer to obtain 250 µM cardiolipin. Add 0, 2, 4, 6, 8, 10, and 12 µl of the 250 µM standard to wells of a white plate to obtain 0, 0.5, 1, 1.5, 2, 2.5 and 3 nmol of cardiolipin per well. Bring up the total volume in these wells to 50 µl with CL Assay buffer.

Notes:

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

b. Immediately store the remaining 5 mM cardiolipin standard at -20 ºC. When stored at -20 ºC, it is stable for several months.

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl:

	Probe Mix
CL Buffer	48 µl
CL Probe	2 µl

Add the probe mix to wells of the 96-well plate containing the samples and standards. Mix well. *Do not add this mix to "Sample background control" wells*.

4. **Measurement:** Incubate at RT for 5 - 10 minutes**.** Record fluorescence at Ex/Em 340/480 nm**.**

5. **Calculations:** Subtract 0 Cardiolipin reading from all readings. Plot the Cardiolipin Standard Curve. If sample background control is higher than 0 cardiolipin, then subtract sample background control reading from sample reading. Apply corrected RFU to Standard Curve to get B nmol Cardiolipin in the sample well

Cardiolipin concentration in sample: C = B / V X D (nmol /ml)

Where **B** = amount of Cardiolipin in the sample well from Standard Curve (nmol)
V = volume of sample added into the well (ml)
D = dilution factor

Cardiolipin molecular weight: 1501 g/mol
Cardiolipin concentrations can also be expressed as nmol Cardiolipin per mg protein.

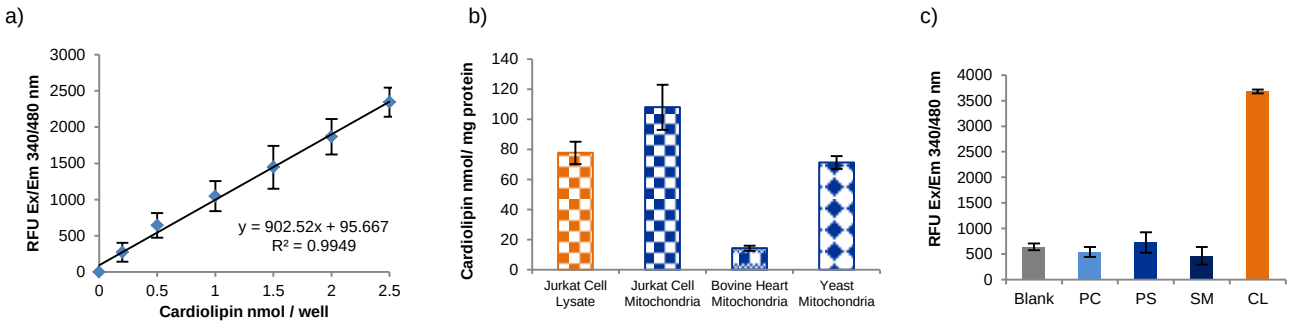


Figure: (a) Cardiolipin standard curve after subtraction of background. (b) Cardiolipin content per mg protein in Jurkat cell lysate, Jurkat cell mitochondria, Bovine heart mitochondria and Yeast Mitochondria (*S. cerevisiae*). (c) Signal from phosphatidylcholine (PC), phosphatidylserine (PS), sphingomyelin (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.

VIII. RELATED PRODUCTS

Mitochondria Isolation Kit for Tissue & Cultured Cells (K288)
Yeast Mitochondria Isolation Kit (K259)
Mitochondria/Cytosol Fractionation Kit (K256)
BCA Protein Assay Kit (K813)
Mitochondrial Complex I Activity Colorimetric Assay Kit (K968)
Mitochondrial Complex III Activity Assay Kit (K520)
Cytochrome Oxidase Activity Colorimetric Assay Kit (K287)
Mitochondrial Permeability Transition Pore Assay Kit (K239)
MitoCapture™ Mitochondrial Apoptosis Detection Fluorometric Kit (K250)

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