Human Cardiolipin (CL) ELISA Kit

Catalog No.: abx150938

Size: 96T

Range: 3.9 - 1000 ng/ml

Sensitivity: < 1.5 ng/ml

Storage: Store standard, detection reagent A, detection reagent B and the 96-well plate at -20°C, and the rest of the kit components at 4° C .

Application: For quantitative detection of CL in human Tissue Homegenates and biological fluids.

Introduction

CL is an important component of the inner mitochondrial membrane, where it constitutes about 20% of the total lipid composition. It can also be found in the membranes of most bacteria. The name 'cardiolipin' is derived from the fact that it was first found in animal hearts. In mammalian cells, but also in plant cells, CL is found almost exclusively in the inner mitochondrial membrane where it is essential for the optimal function of numerous enzymes that are involved in mitochondrial energy metabolism.

Principle of the Assay

This kit is based on a competitive binding enzyme-linked immuno-sorbent assay technology. An antibody specific to CL is pre-coated onto the 96 well plate. A competitive inhibition reaction is launched between biotin labeled CL and unlabeled CL with the pre-coated antibody specific to CL. After washing away the unbound conjugates, avidin conjugated to Horseradish Peroxidase is added to each microplate well and incubated. After TMB substrate solution is added only wells that contain CL will produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow is proportional to the amount of CL captured in the plate. The O.D. absorbance is measured spectrophotometrically at 450nm in a microplate reader, and then the concentration of CL can be calculated.

Kit components

1. One pre-coated 96 well plate
2. Standard: 2 tubes
3. Standard Diluent Buffer: 20 ml
4. Wash Buffer (30X): 20 ml. Dilution: 1:30
5. Diluent A: 12 ml
6. Diluent B: 12 ml
7. Stop solution: 6 ml
8. TMB substrate : 9 ml
9. Detection Reagent A (100X): 120µl
10. Detection Reagent B (100X): 120µl
11. Plate Sealer: 4

Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450nm)
3. Precision pipette and disposable pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5ml tubes
7. Plate cover
8. Absorbent filter papers
9. 100 ml and 1 L volume graduated cylinders

Protocol

A. Preparation of sample and reagents

1. Sample

Isolate the test samples soon after collecting, analyze immediately or store at 4°C for up to 5 days. Otherwise, store at -20°C for up to one month or -80°C for up to two months to avoid loss of bioactivity. Avoid multiple freeze-thaw cycles.

✧ **Biological fluids:** Centrifuge at approximately 1 000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

✧ **Tissue homogenates:** The preparation of tissue homogenates will vary depending upon tissue type. This is just an example. Rinse tissues with ice-cold PBS (0.01 mol/L, ph 7.0-7.2) to remove the excess of blood. Weigh before homogenization. Finely mince tissues and homogenize with a tissue homogenizer on ice in fresh lysis buffer (different lysis buffer are required based on subcellular location of the target protein) and sonicate the cell suspension. Centrifuge the homogenates at 10,000 × g for 5 min to collect the supernatant. Assay immediately or aliquot and store at -20°C.

Note:

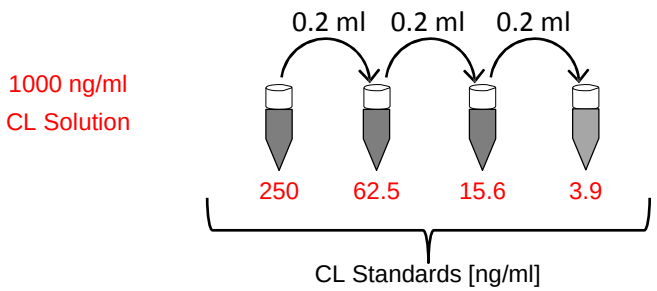
- Please bring sample slowly to room temperature. Sample hemolysis will influence the result.
- Samples must be diluted so that the expected concentration falls within the kit's range. Sample should be diluted in PBS.
- If the sample are not indicated in the manual's applications, a preliminary experiment to determine the validity of the kit will be necessary.
- Fresh samples or recently obtained samples are recommended to prevent protein degradation and denaturalization that may lead to erroneous results.

2. Wash buffer

Dilute the concentrated Wash buffer 30-fold (1/30) with distilled water (i.e. add 20 ml of concentrated wash buffer into 580 ml of distilled water).

3. Standard

Bring samples and all kit components to room temperature. Reconstitute the Lyophilized Standards with **0.5 ml** of Standard Diluent buffer to make the 1000 ng/ml Standard Solution (use within one hour), which also serves as the highest standard. Mix gently and avoid foaming or bubbles. Label 4 tubes with 250 ng/ml, 62.5 ng/ml, 15.6 ng/ml, and 3.9 ng/ml. Aliquot **0.6 ml** of the Standard diluent buffer into each tube. Add **0.2 ml** of the 1,000 ng/ml standard solution into the 1st tube and mix thoroughly. Transfer **0.2 ml** from 1st tube to 2nd tube, mix thoroughly, and so on.



4. Detection Reagent A and B Preparation

Centrifuge Detection Reagent A and B briefly before use. Detection Reagent A and B should be diluted 100-fold with the Diluent A and B respectively and mixed thoroughly. They are sticky solutions therefore pipette with a slow, smooth action to reduce volume errors. The solution should be prepared no more than 2 hours prior to the experiment. The Reconstituted Standard, Detection Reagent A and Detection Reagent B can only be used once.

B. Assay Procedure

Equilibrate the kit components and samples to room temperature before use. It is recommended to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate or triplicate.
2. Aliquot 50 µl of the diluted standards into the standard wells. Aliquot 50 µl Standard Diluent Buffer into the control (zero) wells.
3. Add 50 µl of appropriately diluted sample into the test sample wells. Add the solution at the bottom of each well without touching the side wall. Shake the plate mildly to mix thoroughly.
4. Immediately aliquot 50 µl of Detection Reagent A working solution (if it appears cloudy mix gently until the solution is uniform) to each well. Shake the plate gently to mix thoroughly (a microplate shaker is recommended).
5. Seal the plate with a cover and incubate for 1 h at 37°C.
6. Discard the solution and wash the plate 3 times with wash buffer. Each time let the wash buffer sit for 1-2 min. Do not let the wells dry completely at any time.

Manual Washing: Discard the solution without touching the side walls. Tap the plate on absorbent filter papers or other absorbent material. Fill each well completely with Wash buffer and vortex mildly on ELISA shaker for 2 min. Discard the contents and tap the plate on absorbent filter papers or other absorbent material. Repeat this procedure three times.

Automated Washing: Discard the solution and wash the plate three times with Wash buffer (overfilling wells with the buffer). After the final wash, invert the plate and tap on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 min. Repeat this procedure three times.

7. Aliquot 100 µl of Detection Reagent B working solution into each well, seal and incubate at 37°C for 30 min.
8. Repeat the aspiration/wash process 5 times as explained in step 6.
9. Aliquot 90 µl of TMB Substrate into each well. Seal the plate with a cover and incubate at 37°C for 15-25 min. Avoid exposure to light. The incubation time is for reference use only, the optimal time should be determined by end user. Do not exceed 30 min.
10. Aliquot 50 µl of Stop solution into each well. The color should change to yellow immediately.
11. Read the O.D. absorbance at 450 nm in a microplate reader within 30 min of adding the stop solution.

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The CL concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

C. Precautions

1. Before using the kit, centrifuge the tubes briefly to bring down the contents trapped in the lid.
2. Wash buffer may crystallize and separate. If this happens warm to room temperature and mix gently until the crystals are completely dissolved.
3. Avoid foaming or bubbles when mixing or reconstituting components.
4. It is recommended to measure each standard and sample in duplicate or triplicate.
5. Do NOT let the wells remain uncovered for extended periods between incubation. Once reagents are added to the wells DO NOT let the strips dry at any time during the assay. This can inactivate the biological material on the plate. Incubation time and temperature must be controlled.
6. Do not reuse pipette tips and tubes to avoid cross contamination.
7. Do not use expired components or components from a different kit.
8. The TMB Substrate is light sensitive and should be protected from direct sunlight and UV sources. Store the TMB substrate in the dark and to avoid edge effect of plate incubation for temperature differences it is recommended to equilibrate the TMB substrate for 30 min at room temperature. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

D. Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level CL were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level CL were tested on 3 different plates, 8 replicates in each plate.

CV (%) = SD/meanX100

Intra-Assay: CV<10%

Inter-Assay: CV<12%