Signosis, Inc.
Innovative Plate Assay Solutions

Cholesterol Assay Kit

Catalog # EA-7011 **(For Research Use Only)**

Introduction

The Cholesterol Assay Kit utilizes a series of enzyme reactions to measure cholesterol levels in samples. First, cholesterol esterase is used to hydrolyze cholesterol esters into free cholesterol. Then, cholesterol oxidase is used to convert cholesterol into cholest-4-en-3-one and hydrogen peroxide. The cholesterol level in the sample is determined by quantifying the hydrogen peroxide generated by the enzyme reaction with a fluorogenic probe that can be measured with a spectrophotometer.

Materials Required but Not Provided

- PBS
- 96-well clear microplate for absorbance reading or 96-well black microplate with clear bottom for fluorescence reading
- Microplate reader capable of measuring absorbance at 560 nm or fluorescence at 530nm/590nm

Materials Provided

- 10mM FAD (-80ºC)
- Probe Reagent (-20ºC)
- HRP Reagent (4ºC)
- Triglyceride Standard (RT)
- 1mM Cholesterol Standard (-20ºC)
- 500x CO Enzyme Stock (-80ºC)
- 1x CE Enzyme Stock (-80ºC)

****Spin down small tubes before starting experiment. ****

Plasma Sample Preparation

1. Centrifuge citrated or EDTA-collected blood at 4°C (1,000 x *g* for 10 minutes) to separate plasma from erythrocytes. Alternatively, blood collected without anticoagulant can be centrifuged to collect serum.
2. Transfer the plasma layer to a new tube without disturbing the buffy layer.
3. The plasma may be assayed directly or stored away at -80°C.

Cell Sample Preparation

1. From a 96-well culture plate, detach adherent cells with trypsin. For suspension cells, centrifuge at 1,000 x g for 5 minutes to pellet cells.
2. Wash the cells twice with cold PBS to remove residual media.
3. Resuspend the cells in 1 mL of PBS and homogenize using a tissue grinder or sonicator.
4. Add 2 mL of chloroform and 1 mL of methanol to the homogenized cell sample and mix thoroughly by vortexing for 30 seconds.
5. Add 0.5 mL of ddH_2O to the mixture and vortex again for 30 seconds to induce phase separation.
6. Centrifuge the sample at 1,500 x g for 10 minutes at room temperature to separate the phases.
7. Carefully collect the lower chloroform phase containing the lipids and transfer to a new tube.
8. Vacuum dry the lipid sample until all of the chloroform is evaporated.
9. Reconstitute the dry lipid sample in PBS.
10. The lipid sample may be assayed directly or stored at -80°C.

Tissue Sample Preparation

1. Weigh 100 mg of tissue and place in a tube.
2. Add 1 mL of cold methanol and homogenize using a tissue grinder.
3. Add 2 mL of chloroform to the homogenized tissue sample and mix thoroughly by vortexing for 30 seconds.
4. Add 0.5 mL of ddH_2O to the mixture and vortex again for 30 seconds to induce phase separation.
5. Centrifuge the sample at 1,500 x g for 10 minutes at room temperature to separate the phases.
6. Carefully collect the lower chloroform phase containing the lipids and transfer to a new tube.
7. Vacuum dry the lipid sample until all of the chloroform is evaporated.
8. Reconstitute the dry lipid sample in PBS.
9. The lipid sample may be assayed directly or stored at -80°C.

Cholesterol Measurement

1. Standard curve preparation: Using the provided 1 mM cholesterol standard, prepare a standard curve dilution as described in the table below:

Standard #	1	2	3	4	5	6
Cholesterol Standard Volume (µL)	4	3	2	1	0.5	0
PBS (µL)	36	37	38	39	39.5	40
Total (µL)	40	40	40	40	40	40
Cholesterol Final Concentration (µM)	100	75	50	25	12.5	0

2. Reaction mix preparation: calculate the amount of each reagent needed to make the reaction mix according to the table below.

   For measuring total cholesterol, include the CE Enzyme in the reaction mix. For measuring free cholesterol, exclude the CE Enzyme from the reaction mix.

Component	Reaction Mix (per well/sample)
10mM FAD	0.04 µL
1x CO Enzyme	0.8 µL
1x CE Enzyme	0.16 µL
PBS	x µL
Total	40 µL

3. Dilute the 500x CO enzyme stock to 1x in PBS for the assay. Any unused enzyme stock can be stored at -80ºC for future use.
4. Add 40 µL of reaction mix to each well of the plate.
5. Add 40 µL of sample or standard to each well with reaction mix and mix thoroughly.
6. Cover the plate and incubate at room temperature for 30 minutes.
7. Detection mix preparation: calculate the amount of each reagent needed to make the detection mix according to the table below.

Component	Detection Mix (per well/sample)
Probe Reagent	0.8 µL
HRP Reagent	1.6 µL
PBS	77.6 µL
Total	80 µL

8. Add 80 µL of detection mix to each reaction well in the plate.
9. Cover the plate and incubate at room temperature away from light for 30 minutes.
   Exposure to light will produce background signal in wells
10. For a stronger signal, the plate can be incubated for an additional hour or two away from light.
11. Measure the absorbance of the plate at 560 nm using a plate reader. Alternatively, measure the fluorescence of the plate in a fluorescence plate reader Ex/Em 530nm/590nm.