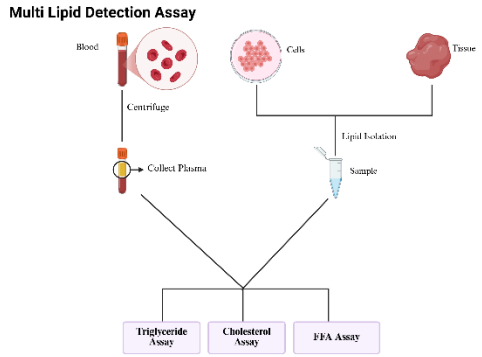
Signosis, Inc.
Innovative Plate Assay Solutions

Multi Lipid Detection Assay Kit (100 Assays)

Catalog # EA-7013 **(For Research Use Only)**

Introduction

Signosis' **Multi Lipid Detection Assay Kit** provides a comprehensive solution for measuring key lipids in blood, cells, or tissue samples in a single, streamlined workflow. This kit enables the detection of **triglycerides, cholesterol,** and **free fatty acids (FFA)** by integrating multiple assays into a single kit.



Principle

Triglyceride Assay

The Triglyceride Assay Kit utilizes a series of enzyme reactions to measure triglyceride levels in samples. First, the triglyceride is hydrolyzed by lipoprotein lipase into glycerol. Then, the glycerol is converted to glycerol-3-phosphate by glycerol kinase. Lastly, the glycerol-3-phosphate is oxidized by glycerol-3-phosphate oxidase, which produces hydrogen peroxide. The triglyceride 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.

Cholesterol Assay

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.

FFA Assay

The Free Fatty Acid (FFA) assay kit utilizes a series of enzyme reactions to measure free fatty acid levels in samples. First, Acyl-Coa synthetase attaches coenzyme A to the FFAs to form Acyl-Coa. Then, Acyl-Coa oxidase reacts with Acyl-Coa to produce hydrogen peroxide. The FFA 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

- KRPG buffer (RT)
- DMSO (RT)
- 100mM ATP (-20ºC)
- 10mM FAD (-80ºC)
- Probe Reagent (-20ºC)
- HRP Reagent (4ºC)
- 450mM Triglyceride Standard (RT)
- 1x Lipase Enzyme Stock (-80ºC)
- 1x GKi Enzyme Stock (-80ºC)
- 1x GPOx Enzyme Stock (-80ºC)
- 1mM Cholesterol Standard (-20ºC)
- 500x CO Enzyme Stock (-80ºC)
- 1x CE Enzyme Stock (-80ºC)
- 10mM FFA Standard (-20ºC)
- Coenzyme A (-20ºC)
- 1x ACS Enzyme Stock (-80ºC)
- 1x ACO 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.

Triglyceride Measurement

1. Standard curve preparation: First, prepare a 225mM triglyceride standard by mixing 10 µL of the provided 450mM triglyceride standard with 10 µL of DMSO. Then, take eight new tubes labeled 1-8 and add 10 µL of DMSO to each tube. Transfer 10 µL of the 225mM standard to the first DMSO tube and mix to make a 112.5mM standard. Then, transfer 10 µL of the 112.5mM standard from the first tube to the second DMSO tube and mix to make a 56.3mM standard. Continue the serial dilutions until the seventh tube is done. Leave the eighth tube untouched as the DMSO negative control.

Standard#	Triglyceride Concentration (mM)
1	225
2	112.5
3	56.3
4	28.1
5	14.1
6	7.0
7	3.5
8	0

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

Component	Reaction Mix (per well/sample)
100mM ATP	0.5 µL
1x Lipase Enzyme	1.5 µL
1x GKi Enzyme	0.05 µL
1x GPOx Enzyme	0.05 µL
KRPG Buffer	47.9 µL
Total	50 µL

3. Any unused enzyme stock can be stored at -80ºC for future use.
4. Add 50 µL of reaction mix to each well of the plate.
5. Add 5 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	0.5 µL
HRP Reagent	1 µL
PBS	48.5 µL
Total	50 µL

8. Add 50 µL of detection mix to each reaction well in the plate. **Be sure to add the detection mix quickly, since the signal begins to develop when the reagents are added. Use a multichannel pipette if possible.**
9. Cover the plate and incubate at room temperature away from light for 10-20 minutes. **Exposure to light will produce background signal in wells**
10. For a stronger signal, the plate can be incubated for another 30-60 minutes 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.

PBS	77.6 µL
Total	80 µL

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

8. Add 80 µL of detection mix to each reaction well in the plate. **Be sure to add the detection mix quickly, since the signal begins to develop when the reagents are added. Use a multichannel pipette if possible.**
9. Cover the plate and incubate at room temperature away from light for 15-30 minutes.
   Exposure to light will produce background signal in wells
10. For a stronger signal, the plate can be incubated for another 30-60 minutes 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.

FFA Measurement

1. Standard curve preparation: Take five new tubes labeled 1-5 and add 10 µL of DMSO to each tube. Transfer 10 µL of the provided 10mM FFA standard to the first DMSO tube and mix to make a 5mM FFA dilution. Then, transfer 10 µL of the 5mM FFA dilution from the first tube to the second DMSO tube and mix to make a 2.5mM dilution. Continue the serial dilutions until the fourth tube is done. Leave the fifth tube untouched as the DMSO negative control.

   Take six new tubes labeled A-F and add 19 µL of PBS to each tube. Transfer 1 µL of the provided 10mM FFA standard to tube A and mix to make a 500 µM FFA standard. Transfer 1 µL from each of the FFA dilutions prepared in tubes 1-5 to tubes B-F and mix to make the rest of the FFA standards.

Standard#	FFA Concentration (µM)
A	500
B	250
C	125
D	62.5
E	31.25
F	0

2. Dissolve the provided 6mg of coenzyme A in 780 µL of ddH_2O to make a 10mM coenzyme A solution. Unused coenzyme A solution can be stored at -20°C for future use.
3. Reaction mix #1 preparation: calculate the amount of each reagent needed to make the reaction mix according to the table below.

Component	Reaction Mix (per well/sample)
100mM ATP	0.2 µL
10mM Coenzyme A	1 µL
1x ACS Enzyme	2 µL
KRPG Buffer	16.8 µL
Total	20 µL

4. Add 20 µL of reaction mix #1 to each well of the plate.
5. Add 20 µL of sample or standard to each well with reaction mix #1 and mix thoroughly.
6. Cover the plate and incubate at 37°C for 30 minutes.
7. Reaction mix #2 preparation: calculate the amount of each reagent needed to make the reaction mix according to the table below.

Component	Reaction Mix (per well/sample)
10mM FAD	0.04 µL
1x ACO Enzyme	0.04 µL
PBS	39.92 µL
Total	40 µL

8. Add 40 µL of reaction mix #2 to each reaction well in the plate.
9. Cover the plate and incubate at 37°C for 30 minutes.
10. 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

11. Add 80 µL of detection mix to each sample or standard well in the plate. **Be sure to add the detection mix quickly, since the signal begins to develop when the reagents are added. Use a multichannel pipette if possible.**
12. Cover the plate and incubate at room temperature away from light for 30 minutes.
    Exposure to light will produce background signal in wells
13. For a stronger signal, the plate can be incubated for 1-2 hours or longer away from light.
14. 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.