Signosis, Inc.
Innovative Plate Assay Solutions

Triglyceride Assay Kit

Catalog # EA-7010 **(For Research Use Only)**

Introduction

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.

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 (-20°C)
- Probe Reagent (-20°C)
- HRP Reagent (4°C)
- Triglyceride Standard (RT)
- 1x Lipase Enzyme Stock (-80°C)
- 1x GK Enzyme Stock (-80°C)
- 1x GPO Enzyme Stock (-80°C)

****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 0.5x triglyceride standard by mixing 10 µL of the provided 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 0.5x standard to the first DMSO tube and mix to make a 0.25x standard. Then, transfer 10 µL of the 0.25x standard from the first tube to the second DMSO tube and mix to make a 0.125x standard. Continue the serial dilutions until the seventh tube is done. Leave the eight tube untouched as the DMSO negative control.

Standard#	Triglyceride Concentration (X)
1	0.25
2	0.125
3	0.0625
4	0.03125
5	0.01563
6	0.007813
7	0.003906
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 GK Enzyme	0.05 µL
1x GPO 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.