Signosis
BioSignal Capture

Triglyceride Assay Kit

Catalog Number 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 provided with the kit

- KRPG buffer (RT)
- Triglyceride Standard (-20ºC)
- Glycerol Enzyme Reagent (-20ºC)

Material required but not provided

- 96-well clear microplate
- Microplate reader capable of measuring absorbance at 560 nm

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.
11. Dilute the lysates 1:10 with KRPG buffer before beginning assay.

Triglyceride Measurement

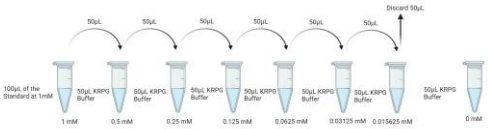
1. Prepare a triglyceride standard curve in a 96-well clear plate using an 8-well serial dilution. In the first well, dilute 20 µL of the 10 mM triglyceride stock solution in 80 µL of KRPG buffer to make a 1 mM triglyceride standard. Next, add 50 µL of KRPG buffer to the next 7 wells. Then, transfer 50 µL of the first well to the next well to make a two-fold dilution. Perform six additional two-fold serial

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.
10. Dilute the lysates 1:10 with KRPG buffer before beginning assay.

Triglyceride Measurement

1. Prepare a triglyceride standard curve in a 96-well clear plate using an 8-well serial dilution. In the first well, dilute 20 µL of the 10 mM triglyceride stock solution in 80 µL of KRPG buffer to make a 1 mM triglyceride standard. Next, add 50 µL of KRPG buffer to the next 7 wells. Then, transfer 50 µL of the first well to the next well to make a two-fold dilution. Perform six additional two-fold serial dilutions and leave the last, 8th well untouched as the blank buffer well.



2. Add 50 µL of plasma or serum samples to each well of the plate. Dilute samples in KRPG buffer if necessary.
3. Prepare enzyme detection solution by mixing the enzyme reagent in KRPG buffer.
4. Add 50 µL of the enzyme detection solution to each sample or standard in the 96-well plate.
5. Incubate the plate at room temperature for 1 hour.
6. Measure the absorbance of the plate at 560 nm using a plate reader.