CREATIVE BIOMART®
Assay Kit

Triglyceride Assay Kit

Product Information

Common Name

Triglyceride

Cat.No. Kit-2172

Product Overview

Triglyceride Assay Kit provides a simple, reproducible, and sensitive tool for assaying triglycerides in plasma, serum, cell lysates, and tissue homogenate samples. The Triglyceride Colorimetric Assay uses the enzymatic hydrolysis of the triglycerides by lipase to glycerol and free fatty acids. The glycerol released is subsequently measured by a coupled enzymatic reaction system. The glycerol formed in reaction 1 is phosphorylated to glycerol- 3-phosphate in a reaction catalyzed by glycerol kinase. The glycerol-3- phosphate is oxidized by glycerol phosphate oxidase producing dihydroxyacetone phosphate and hydrogen peroxide. Peroxidase catalyzes the redox-coupled reaction of H2O2 with 4-aminoantipyrine (4-AAP) and N-Ethyl-N-(3-sulfopropyl)- m-anisidine (ESPA), producing a brilliant purple color. The absorbance is measured at 540 nm.

Description

Triglycerides are water-insoluble lipids consisting of three fatty acids esterified to a glycerol backbone. Triglycerides are transported in the blood as core constituents of all lipoproteins, but are major components of triglyceride-rich chylomicrons and very low-density lipoproteins (VLDL). A major source of triglycerides is dietary fat. Dietary fats are hydrolyzed in the gut into free fatty acids and mono- and diglycerides and then transported through the intestinal villi. After absorption through the gut, they are resynthesized into new triglycerides and assembled into chylomicrons. Triglycerides are rapidly hydrolyzed in the capillary beds by lipoprotein lipase, releasing glycerol and free fatty acids, which are absorbed by adipose tissue for storage. When required, lipases hydrolyze triglycerides from adipose tissue into fatty acids and glycerol, which enter the blood stream. Fatty acids are oxidized in the mitochondria and peroxisomes to produce energy. Triglycerides play an important role in metabolism by containing more than twice as much energy as carbohydrates and proteins.

Synonyms

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Triglyceride Colorimetric Assay Kit; TG Assay Kit

Size

96 wells

Kit Components

Triglyceride Standard: 1 vial/400 µl
Standard Diluent Assay Reagent (5X): 1 vial/12 ml
Sodium Phosphate Assay Buffer: 1 vial/4 ml
Triglyceride Enzyme Mixture: 1 vial
96-Well Cover Sheet: 1 cover
96-Well Solid Plate (Colorimetric Assay): 1 plate

Preparation

Reagent Preparation:
1. Triglyceride Standard - The vial contains 400 µl of a 1,000 mg/dl solution of Triglyceride Standard. It is ready to use as provided to prepare the standard curve. Sufficient Triglyceride Standard is provided to prepare three standard curves.
2. Standard Diluent Assay Reagent (5X) - The vial contains 12 ml of a (5X) salt solution. Prior to use, dilute the contents of the vial with 48 ml of HPLC-grade water. This diluted Standard Diluent solution is used to prepare the triglyceride standards and may be stored for six months at room temperature until it is ready for use.
3. Sodium Phosphate Assay Buffer - The vial contains 4 ml of 250 mM sodium phosphate buffer, pH 7.2. Prior to use, dilute the contents of the vial with 16 ml of HPLC-grade water. This diluted buffer (50 mM sodium phosphate, pH 7.2) is used to prepare the triglyceride enzyme solution. The Assay Buffer may be stored for at least six months at room temperature until it is ready for use.
4. Triglyceride Enzyme Mixture - The vial contains a lyophilized enzyme mixture. Reconstitute the contents of the vial with 1 ml of HPLC-grade water. Transfer the reconstituted solution to a 15 ml centrifuge tube wrapped in aluminum foil. Add 14 ml of the diluted Assay Buffer to the reconstituted solution and mix by inversion.
NOTE: A portion of the 14 ml should be used to rinse any residual solution from the vial. This solution is now ready to use in the assay. If the entire solution is not used at one time, the solution should be

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stored at 4°C. Do NOT Freeze! The solution is stable for one month when stored at 4°C; a slight pink discoloration may occur but will have no affect on the assay performance.

Sample Preparation:

Plasma

Typically, normal human plasma has triglyceride concentrations in the range of 40-160 mg/dl (male) or 35-135 mg/dl (female).

1. Collect blood using an anticoagulant such as heparin, EDTA, or citrate.
2. Centrifuge the blood at 700-1,000 x g for 10 minutes at 4°C. Pipette off the top yellow plasma layer without disturbing the white buffy layer. Store plasma on ice. If not assaying the same day, freeze at -80°C. The plasma sample will be stable for one month while stored at -80°C.
3. Plasma does not need to be diluted before assaying.

Serum

Typically, normal human serum has triglyceride concentrations in the range of 40-160 mg/dl (male) or 35-135 mg/dl (female).

1. Collect blood without using an anticoagulant.
2. Allow blood to clot for 30 minutes at 25°C.
3. Centrifuge the blood at 2,000 x g for 15 minutes at 4°C. Pipette off the top yellow serum layer without disturbing the white buffy layer. Store serum on ice. If not assaying the same day, freeze at -80°C. The serum sample will be stable for one month while stored at -80°C.
4. Serum does not need to be diluted before assaying.

Cell Lysates

1. Collect cells (~18 x 106 cells) by centrifugation (i.e., 1,000-2,000 x g for 10 minutes at 4°C). For adherent cells, do not harvest using proteolytic enzymes; rather use a rubber policeman.
2. Resuspend the cell pellet in 1-2 ml of cold diluted Standard Diluent.
3. Sonicate the cell suspension 20X at one second bursts.
4. Centrifuge cell suspension at 10,000 x g for 10 minutes at 4°C.
5. Remove the supernatant and store on ice. If not assaying on the same day, freeze at -80°C until use. The sample will be stable for at least one month.
6. Before assaying, further dilute the samples 1:2-1:3 with diluted Standard Diluent.

Tissue Homogenates

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1. Weigh tissue and then mince into small pieces.
2. Homogenize 350-400 mg of minced tissue in 2 ml of the diluted Standard Diluent containing protease inhibitors of choice.
3. Centrifuge at 10,000 x g for 10 minutes at 4°C.
4. Transfer the entire supernatant to another tube. Store the supernatant on ice. If not assaying the same day, freeze at -80°C. The sample will be stable for one month while stored at -80°C.
NOTE: When centrifuged at high speeds some tissue homogenates, such as liver, will leave a layer of insoluble fat at the top of the centrifuge tube. Be sure to include this layer when transferring the supernatant.
5. Typically, tissue samples require dilutions of at least 1:5 or greater. Dilute the samples using the diluted Standard Dilutent before assaying.

Assay Protocol

General Information

- All reagents except samples must be equilibrated to room temperature before beginning the assay.
- The final volume of the assay is 160 µl in all wells.
- The incubation temperature is at room temperature.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended that the standards and samples be assayed at least in duplicate.
- Monitor the absorbance at 530-550 nm using a plate reader.

Standard Preparation

Take eight clean test tubes and label them 1-8. Add 200 µl of the diluted Standard Diluent to tubes 2-8. Add 400 µl of diluted Standard Diluent to tube 1. Add 100 µl of Triglyceride Standard to tube 1 and mix thoroughly. The concentration of Tube 1 is 200 mg/dl (2.26 mmol/L), from which serial dilutions will be made. Serially dilute the triglycerides by removing 200 µl from tube 1 and adding it to tube 2; mix thoroughly. Next, remove 200 µl from tube 2 and place it into tube 3; mix thoroughly. Repeat this process for tubes 4-7. Tube 8 only has diluted Standard Diluent and is used as the blank. We recommend that you store these diluted standards for no more than one to two hours.

Table 1. Preparation of Triglyceride Standards

Tube Triglyceride Concentration (mg/dl)

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1   200
2   100
3   50
4   25
5   12.5
6   6.25
7   3.125
8   0
Performing the Assay
1. Triglyceride Standard Wells - Add 10 µl of standard (tubes 1-8) per well in the designated wells on the plate.
2. Sample Wells - Add 10 µl of sample to two or three wells.
NOTE: The amount of sample added to the well should always be 10 µl.
3. Initiate the reaction by adding 150 µl of diluted Enzyme Buffer solution to each well.
4. Carefully shake the microwell plate for a few seconds to mix. Cover with the plate cover.
5. Incubate the plate for 15 minutes at room temperature.
6. Read the absorbance at 530-550 nm using a plate reader

Analysis

Calculations
1. Calculate the average absorbance of each standard and sample.
2. Subtract the absorbance value of standard 8 (0 mg/dl) from itself and all other values (both standards and samples). This is the corrected absorbance.
3. Graph the corrected absorbance values (from step 2 above) of each standard as a function of the final triglyceride concentration (mg/dl).
4. Calculate the values of triglyceride samples using the equation obtained from the linear regression of the standard curve by substituting the corrected absorbance values for each sample into the equation.
Triglycerides (mg/dl) = [(Corrected absorbance) - (y-intercept)]/Slope

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