Triglyceride Assay Kit

Catalog No.: abx298852

Size: 100 Assays

Storage: Store the Enzyme and Dye Reagent at -20°C and all other kit components at 4°C.

Application: For quantitative detection of Triglyceride concentrations in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants, urine and other biological fluids.

Detection Range: 0.1 mmol/L – 5 mmol/L

Introduction: Triglycerides are esters derived from esters and three fatty acids. They are the main component of animal and vegetable fats. They play an important role in many metabolic processes. High blood triglyceride concentrations are implicated in several diseases, such as atherosclerosis, stroke and coronary heart disease.

Abbexa's Triglyceride Assay Kit is a quick, convenient, and sensitive method for measuring and calculating triglyceride concentrations. Triglycerides are hydrolyzed by the enzyme lipase to produce glycerol and free fatty acids. The glycerol released is measured by a coupled enzymatic reaction system, with a colorimetric readout at 500 nm.

Kit components

1. 96 well microplate
2. Diluent Buffer: 20 ml
3. Enzyme: 1 vial
4. Dye Reagent: 1 vial
5. Standard (5 mmol/L): 1 ml
6. Plate Sealer: 3

Materials Required But Not Provided

1. Microplate reader (500 nm) and incubator
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Heptane
6. Isopropanol
7. Timer
8. Ice
9. Sonicator
10. Mortar

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Enzyme Solution**
  Add 9 ml of Diluent Buffer into the Enzyme vial and mix thoroughly to prepare the Enzyme Solution. Ensure that the Enzyme has completely dissolved prior to use.

- **Dye Reagent Solution**
  Add 10 ml of Diluent Buffer into the Dye Reagent vial and mix thoroughly to prepare the Dye Reagent Solution. Ensure that the Dye Reagent has completely dissolved prior to use.

- **Assay Buffer Solution**
  Add 35 ml of Heptane to 65 ml of Isopropanol to prepare 100 ml of Assay Buffer Solution. Mix thoroughly.

2. Sample

- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant and add 1 ml of Assay Buffer Solution for every 5,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Centrifuge at 10,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of Assay Buffer Solution on ice. Centrifuge at 10,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

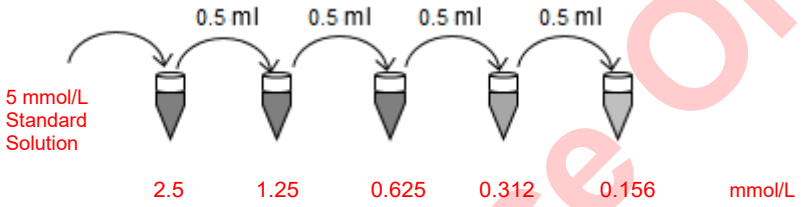
- **Serum and plasma samples**
  Serum and plasma samples can be used directly.

  Triglyceride concentrations can vary over a wide range depending on the sample type and population. If the expected concentration is unknown, a preliminary experiment is recommended to determine if sampled dilutions are required to bring the measured concentrations within the detection range of the kit.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

1. Label 5 tubes with 2.5 mmol/L, 1.25 mmol/L, 0.625 mmol/L, 0.312 mmol/L and 0.156 mmol/L. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 5 mmol/L Standard Solution to the 1st tube, and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Set the sample, standard and blank wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 10 µl of sample to the sample wells.
4. Add 10 µl of prepared standards to the standard wells.
5. Add 10 µl of distilled water to the blank wells.
6. Add 90 µl of Enzyme Solution to each well.
7. Add 100 µl of Dye Reagent Solution to each well.
8. Tap the plate gently to mix. Cover the plate with a Plate Sealer, then incubate at 37°C for 10 minutes.
9. Read and record absorbance at 500 nm.

C. Calculations

Triglyceride concentration per mg of protein:

$$\mathbf{Triglyceride\ (\mu mol/mg)} = \frac{\mathbf{C_{Standard} \times V_{Standard}}}{\mathbf{V_{Sample} \times C_{Protein}}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{5}}{\mathbf{C_{Protein}}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

Triglyceride concentration per g of sample:

$$\mathbf{Triglyceride\ (\mu mol/g)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{V_{Sample} \times W}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{5}}{\mathbf{W}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

Triglyceride concentration per 10^4 cells or bacteria:

$$\mathbf{Triglyceride\ (\mu mol/10^4\ cells)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{V_{Sample} \times N}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{5}}{\mathbf{N}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

Triglyceride concentration per ml of serum or plasma:

$$\mathbf{Triglyceride\ (\mu mol/ml)} = \frac{\mathbf{C_{Standard} \times V_{Standard}}}{\mathbf{V_{Sample}}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \mathbf{5} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

where:

$\mathbf{C_{Protein}}$	Concentration of protein (in mg/ml)
$\mathbf{C_{Standard}}$	Concentration of highest standard (5 mmol/L = 5 µmol/ml)
W	Weight of the sample (in g)
N	Number of cells or bacteria (× 10^4)
$\mathbf{V_{Assay}}$	Volume of Assay Buffer Solution (1 ml)
$\mathbf{V_{Sample}}$	Volume of sample (10 µl = 0.01 ml)
$\mathbf{V_{Standard}}$	Volume of standard (10 µl = 0.01 ml)