ELK Biotechnology

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Triglyceride Assay Kit Instruction

CAT/NO.: BC010 Microplate Method

1. Reagent Composition & Preparation

Reagents	Format	Compositions	Concentration	Storage Environment
Enzyme Reagent	1 Bottle of 25mL	Tris-HCl Buffer	100mM	2-8℃ Avoid Light
		Lipase	≥3000U/L	
		ATP	0.5mM	
		Glycerol Kinase	≥1000U/L	
		Glycerol-3-phosphate Oxidase	≥5000U/L	
		Peroxidase	≥1000U/L	
		4 Amino-antipyrene (4-AAP)	1.4mM	
		p-Chlorophenol	3mM	
Standard Solution	1 Bottle of 1mL	Triglycerol	2.26mM	
One 96-well Flat-Bottom Microtiter Plate			Preserved at Room Temperature	

2. Principle of Measurement

$$\text{Triglyceride} + H_2O \xrightarrow{Lipase} Glycerol + Fatty\ Acid$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{Glycerol\ Kinase} Glycerol-3-Phosphate + ADP$$

$$\text{Glycerol}-3-\text{Phosphate} + O_2 \xrightarrow{Glycerol\text{-}3\text{-}Phosphate\ Oxidase} Dihydroxyacetone\ Phosphate + H_2O_2$$

$$H_2O_2 + 4-\text{AAP} + \text{p}-\text{Chlorophenol} \xrightarrow{Peroxidase} \text{Quinone (Red Compounds)}$$

The optical spectrum absorbance at certain wavelength is proportion to the concentration of Quinone compound in the solution. This concentration relates to the Triglyceride content of the sample. Thus the Triglyceride's content can be determined via the absorbance difference between the standard tube and the sample tube according to the absorbance of the blank tube.

3. Procedures of Measurement

I. Sample Pre-Treatment

1. Serum (Plasma): Can be used for further measurement directly. The serum should be diluted with physiological saline if the concentration of triglyceride in serum exceeds the concentration range allowed.
2. Medium Sample: The medium should be extracted and separated by centrifugation at 2500rmp for 10 minutes. The supernatant is desired.
3. Tissue Sample: The sample is weighed precisely and for every 1g of the sample, 9mL homogenate medium should be mixed in ice water bath. The homogenate treated with centrifugation at 2500rpm for 10 minutes would be separated and supernatant is extracted for further use.

   Note 1. The homogenate medium is 0.1mM phosphate buffer (pH=7.4) or physiological saline for tissue samples without high fat part.

   Note 2. For high fat or partial high fat sample, the homogenate medium used is ethyl alcohol absolute.
4. Sample Cells

   i. Cell Harvesting

      Cell suspension is extracted and centrifuged with 1000rpm for 10 minutes. Supernatant should be discarded and isotonic buffer (0.1M, pH 7-7.4 phosphate buffer recommended) is used to wash the precipitates for once or twice. The suspension is separated by centrifugation for 10 minutes and the precipitates should be kept for further treatment.

   ii. Cell Disruption

      0.2 to 0.3 mL homogenate medium should be added and homogenized (0.1M, pH 7-7.4 phosphate buffer or physiological saline recommended). Cells can be disrupted with ultrasonic disruptor in ice water bath. The ultrasonic disruptor is 300W and the disruption is repeated for 3-5 times with the period of 3-5 seconds and interval of 30 seconds. The homogenate can be measured directly. Also, Lysate can be used directly for the later measurement (Triton X-100, 1-2% recommended for the splitting period of 30-40 minutes).

   Note that the cell density in the sample is recommended to be no less than 1 million/mL. Microscope can be used to observe the completeness of cell disruption.

II. Measurement Procedures

Operated in 96-well Microtiter Plate with Microplate Reader			
Compositions (μL)	Blank	Standard	Sample
Distilled Water	2.5		
2.26mM Standard Solution		2.5	
Sample			2.5
Enzyme reagent	250	250	250

The solution in the tube is mixed and incubated for 10 minutes at 37℃. Spectrum

absorbance (OD) at 500nm wavelength is measured.

Operated by Automatic Biochemical Analyzer			
Sample	2.5μL	Enzyme Reagent	250μL
The solution is mixed and incubated for 10 minutes at 37℃. Analyzer should be adjusted with enzyme reagent and distilled water. Absorbance A is measured.			
Main Wavelength	510nm	Reaction Type	End-Point Method
Reaction Direction	Forward		

4. Calculation Formula and Example

1. Serum or other Liquid Sample Formula

   For Microplate Reader:

$$\underset{mM}{Triglyceride\ Concentration} = \frac{OD_{sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{(2.26mM)}$$

   For Automatic Biochemical Analyzer:

$$\underset{mM}{Triglyceride\ Concentration} = \frac{A_{sample}}{A_{Standard}} \times \frac{C_{Standard}}{(2.26mM)}$$

   Example: 2.5mL human serum was measured and the absorbance values are 0.0573, 0.2340 and 0.1283 for blank, standard and sample solution. The Triglyceride concentration is shown below

$$\underset{mM}{Triglyceride} = \frac{OD_{sample} - OD_{Blank}}{OD_{Standard} - OD_{blank}} \times \frac{C_{Standard}}{(2.26mM)} = \frac{0.1283 - 0.0573}{0.2340 - 0.0573} \times 2.26mM = 0.9081\text{mM}$$

   Example: 2.5μL blood plasma was measured and the absorbance values are 0.0573, 0.2340 and 0.1030 in accordance to the sequence listed above.

$$\underset{Mm}{Triglyceride} = \frac{OD_{sample} - OD_{Blank}}{OD_{Standard} - OD_{blank}} \times \frac{C_{Standard}}{(2.26mM)} == \frac{0.1030 - 0.0573}{0.2340 - 0.0573} \times 2.26mM = 0.5845\text{mM}$$

2. Formula for Samples of Tissue or Cell

   For microplate reader:

$$\underset{mmol/g}{Triglyceride} = \frac{OD_{sample} - OD_{Blank}}{OD_{Standard} - OD_{blank}} \times \frac{C_{Standard}}{(2.26mM)} \div C(ggprot/L)$$

   For Automatic Biochemical Analyzer:

$$\underset{mmol/g}{Triglyceride} = \frac{A_{sample}}{A_{Standard}} \times \frac{C_{Standard}}{(2.26mM)} \div C(gprot/L)$$

Example: 2.5 homogenate from mouse's liver was measured and the absorbance values are 0.0580, 0.2352 and 0.1583 in accordance to the sequence listed above. Also, the protein concentration is measured to be 12.0121 g/L

$$\frac{Triglyceride}{mmol/gprot} = \frac{OD_{sample} - OD_{Blank}}{OD_{Standard} - OD_{blank}} \times \frac{C_{Standard}}{(2.26mM)} \div \rho(gprot/L)$$

$$= \frac{0.1583 - 0.0580}{0.2352 - 0.0580} \times 2.26 \div 12.0121 = 0.1065 mmol/g$$

5. Product Description

This assay kit is produced based on the GPO-PAP method and is used to measure the content of triglyceride out of the body. This kit can be applied to different types of microplate reader, semi-automatic or automatic biochemical analyzer.

6. Performance Index

1. Blank tube absorbance value≤0.200.
2. Concentration range 0-9.04mM with r^2>0.995.
3. Accuracy with the relative deviation≤10%
4. The absorbance value A is around 0.2200-0.2900 for the 2.26mM Standard solution.
5. The precision of the measurement is ≤5.5% and coefficient of variation≤ 8.0%.
6. The reagent in the original package should be preserved at 2-8℃ without light-struck and is stable within 12 months. The unsealed package can be preserved for one month in the same environment.

7. Notes

1. This product is designed for research. Usage for clinical trials or oral taken is prohibited.
2. Please dilute the sample with physiological saline when the concentration exceeds the range permitted.
3. The reagent can be contaminated by glucose or cholesterol assay reagent.
4. The amount of reagent and sample tested can be adjusted according to the instructions of the automatic biochemical analyzer with the ratio of 100:1.