ELK Biotechnology
For Research Use Only

Total-Cholesterol Assay Kit

CAT/NO.：BC007 Microplate method

I. Reagent Compositions and Preparation

Reagents	Format	Compositions	Concentration	Storage Environment
Enzyme Reagent	1 Bottle of 25mL	Good's Buffer, pH6.7	50mM	2-8℃ Avoid Light
		Phenol	5mM	
		4 Amino-Antipyrene (4-AAP)	0.3mM	
		Cholesteryl Esterase	≥50,000U/L	
		Cholesterol Oxidase	≥25,000U/L	
		Peroxidase	≥1,300U/L	
Standard Solution	1 vial	Cholesterol	7.01mM	
One 96-well Flat-Bottom Microtiter Plate			Preserved at Room Temperature	
It comes with a disposable 96-hole plate				

II. Principle of Measurement

$$Cholesteryl\ Ester + H_2O \xrightarrow{Cholesteryl\ Esterase} Cholesterol + Fatty\ Acid$$

$$Cholesterol + O_2 \xrightarrow{Cholesterol\ Oxidase} \text{Cholest} - 4 - \text{en} - 3 - \text{one} + H_2O_2$$

$$H_2O_2 + 4 - AAP + Phenol \xrightarrow{Peroxidase} Quinone\ (Red) + H_2O$$

The red quinone concentration is proportional to the optical density (OD) absorbed at certain wavelength. Thus the cholesterol concentration can be calculated based on the OD values.

III. Procedures

1. Pretreatment

i. **Serum/Plasma Sample:** Can be measured directly or diluted with physiological saline were the results too high

ii. **Culture Medium**: Extract the medium and centrifuge at 1,000 rpm for 10 min. Extract the supernatant.

iii. **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.

iv. Sample Cells

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.

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.

2. 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

Mix thoroughly and warm at 37°C for 10 min. Record the OD value of each well at 510 nm.

Operated by Automatic Biochemical Analyzer			
Sample	2.5μL	Enzyme Reagent	250μL
The solution is mixed and incubated for 10 minutes at 37°C. Analyzer should be adjusted with enzyme reagent and distilled water. Absorbance A is measured of each			

tube.			
Main Wavelength	510nm	Reaction Type	End-Point Method
Reaction Direction	Forward		

IV. Calculation Formula and Examples

1. Serum/Plasma Sample

For Microplate Reader: $\frac{Cholesterol\ Conc.}{mM} = \frac{OD_{sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{(5.17mM)}$

For Automatic Biochemical Analyzer: $\frac{Cholesterol\ Conc.}{mM} = \frac{A_{sample}}{A_{Standard}} \times \frac{C_{Standard}}{(5.176mM)}$

Example

a. Human plasma was measured. The OD values were 0.0443, 0.2344 and 0.1724 respectively.

$$\frac{Cholesterol\ Conc.}{mM} = \frac{OD_{sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{(5.17mM)} = \frac{0.1724 - 0.0443}{0.2344 - 0.0443} \times 5.17$$
$$= 3.484mM$$

b. Rat serum was measured with the OD values equal to 0.0443, 0.2344 and 0.1011 respectively.

$$\frac{Cholesterol\ Conc.}{mM} = \frac{OD_{sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{(5.17mM)} = \frac{0.1011 - 0.0443}{0.2344 - 0.0443} \times 5.17$$
$$= 1.545mM$$

2. Tissue and Cell Sample

For Microplate Reader:

$$\frac{Cholesterol\ Conc.}{mmol/gprot} = \frac{OD_{sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{(5.17mM)} \div \frac{C_{Protein}}{gprot/L}$$

For Automatic Biochemical Analyzer:

$$\frac{Cholesterol\ Conc.}{mmol/gprot} = \frac{A_{sample}}{A_{Standard}} \times \frac{C_{Standard}}{(5.17mM)} \div \frac{C_{Protein}}{gprot/L}$$

Example

a. 10% mouse hepatic homogenate was prepared and measured with OD values equal to 0.0452, 0.2350 and 0.1038 respectively. Also the protein concentration of the homogenate was 12.0121 g/L.

$$\frac{Cholesterol\ Conc.}{mmol/gprot} = \frac{OD_{sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{(5.17mM)} \div \frac{C_{Protein}}{gprot/L}$$
$$= \frac{0.1038 - 0.0452}{0.2350 - 0.0452} \times 5.17 \div 12.0121 = 0.1329mmol/g$$

V. Product Description

This assay kit is produced based on the COD-PAP method and is used to measure the content of cholesterol in vitro. This kit can be applied to different types of microplate reader, semi-automatic or automatic biochemical analyzer.

VI. Performance Index

1. Blank tube absorbance value≤0.100.
2. Concentration range 0-10.34mM with r^2>0.995.
3. Accuracy with the relative deviation≤3% and relative range≤5%
4. Stability: Sealed kit with the term of validity of 12 months at 2-8°C without light struck. Unsealed kit is stable in 3 months with the same condition.

VII. 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.

VIII. References

Li Jianzhai, Current Situation of the Determination of Serum/Plasma Cholesterol. Journal of Chinese Medical Association, 1982-1, P36.
Ye Ying Wu, Wang Yu San. National Clinical Laboratory Procedures. Third Edition, Southeast University Press, 2006, P479.