ELK Biotechnology

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Low Density Lipoprotein – Cholesterol (LDL-C) Assay Kit

Microplate Method

CAT/NO.: BC028 96T/25mL

1. Reagents Composition and Preparation

Reagents	Format	Composition	Concentration	Storage Condition
Reagent I	1 Bottle× 18mL	Good's Buffer	50mmol/L	2-8℃ Avoid Light
		4-Amino-Antipyrene (4-AAP)	0.2mmol/L	
		Magnesium Chloride Hexahydrate	15mmol/L	
		Cholesterol Oxidase (CO)	≥3Ku/L	
		Cholesteryl Esterase (CE)	≥2kU/L	
		Peroxidase	≥5kU/L	
		Surfactant 1	0.1%	
Reagent II	1 Bottle×6mL	Good's Buffer	50mmol/L	
		N-Ethyl-N-(2-Hydroxy-3-Sulfopropyl)-3-Methylaniline Sodium Salt (Toos)	1.0mmol/L	
		Preservative	100mg/L	
		Surfactant 2	0.1%	
Standard	1 Bottle of 1mL	Cholesterol	Labelled	
One 96-well Flat-Bottomed Microtiter Plate Attached			Preserved at Room Temperature	

2. Principle of Measurement

1. First Reaction

$$\begin{matrix} HDL \\ VLDL \\ ULDL \end{matrix} \xrightarrow{Surfactant\ I} Micronized\ Lipoprotein \xrightarrow{CE,CO} H_2O_2$$

$$H_2O_2 + 4 - AAP \xrightarrow{Peroxidase} Compound\ (Colorless)$$

$$LDL \xrightarrow{Surfactant\ I} Micronized\ LDL$$

2. Second Reaction

$$LDL \xrightarrow{Surfactant\ II} Micronized\ Lipoprotein \xrightarrow{CE,CO} H_2O_2$$

$$H_2O_2 + 4 - AAP + Toos \xrightarrow{Peroxidase} Compound\ (Colored)$$

Note 1. HDL, VLDL and ULDL represents high-density lipoprotein, very low-density lipoprotein and chylomicrons respectively

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 LDL-C in serum exceeds the concentration range allowed.
2. Growth Medium Sample: Sample medium should be extracted and separated by centrifugation at 2,500rmp for 10 minutes. Supernatant is desired.
3. Tissue Sample: Samples should be weighed precisely and for every 1g of the sample, 9mL homogenate medium is mixed with sample 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 0.1mmol/L 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 (0.1mol/L, pH 7-7.4 phosphate buffer recommended) is used to wash the precipitates for once or twice. Suspension is separated by centrifugation for 10 minutes and precipitates should be kept for further treatment.

   ii. Cell Disruption

   0.2 to 0.3 mL homogenate medium should be added and homogenized (0.1mol/L, 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 cell density in sample is recommended to be no less than 1 million/ml. Microscope can be used to observe the completeness of cell disruption.

Measurement Procedures

Operated in 96-well Microtiter Plate with Microplate Reader			
Composition (μL)	Blank	Standard	Sample
Distilled Water	2.5		
1.8mmol/L Standard		2.5	
Sample			2.5
Reagent 1	180	180	180
Incubated at 37℃ for 5 min. Spectrum absorbance A_1 at 600nm for each tube should be recorded.			
Reagent 2	60	60	60
Incubated at 37℃ for 5 min. Spectrum absorbance A_2 at 600nm for each tube should be recorded.			

Operated by Automatic Biochemical Analyzer			
Sample/Water	2.5μL	Reagent 1	180μL
Incubated at 37℃ for 5 min. Spectrum absorbance A_1 at 600nm for each tube should be recorded.			
Reagent 2	60μL		
Incubated at 37℃ for 5 min. Spectrum absorbance A_2 at 600nm for each tube should be recorded.			
Main Wavelength	600nm	Reaction Type	End-Point Assay
Reaction Direction	Forward		

4. Calculation Formula and Example

1. Serum or other Liquid Sample

   For Microplate Reader:

$$\frac{LDL-C}{mM} = \frac{(A_{2,Sample} - A_{1,Sample}) - (A_{2,Blank} - A_{1,Blank})}{(A_{2,Standard} - A_{1,Standard}) - (A_{2,Blank} - A_{1,Blank})} \times C_{Standard}$$

   For Automatic Biochemical Analyzer:

$$\frac{LDL-C}{mM} = \frac{A_{2,Sample-}A_{1,Sample}}{A_{2,Standard} - A_{1,Standard}} \times C_{Standard}$$

Example: 2.5μL human serum was measured and absorbance values are listed below:

$A_{1,Blank} = 0.0533, A_{2,Blank} = 0.0576,\ A_{1,Standard} = 0.0842,\ A_{2,Standard} = 0.5418,$ $A_{1,Sample} = 0.0682\ and\ A_{2,Sample} = 0.2083$. The standard reagent's concentration is 4.3mM.

The LDL-C concentration is

$$\frac{LDL-C}{mM} = \frac{(A_{2,Sample} - A_{1,Sample}) - (A_{2,Blank} - A_{1,Blank})}{(A_{2,Standard} - A_{1,Standard}) - (A_{2,Blank} - A_{1,Blank})} \times C_{Standard}$$

$$= \frac{(0.2083 - 0.0682) - (0.0576 - 0.0533)}{(0.5418 - 0.0842) - (0.0576 - 0.0533)} \times 4.3mM = 1.288mM$$

Example: 2.5μL serum taken from rat was measured and absorbance values are listed below:

$A_{1,Blank} = 0.0533, A_{2,Blank} = 0.0576,\ A_{1,Standard} = 0.0842,\ A_{2,Standard} = 0.5418,$ $A_{1,Sample} = 0.0605\ and\ A_{2,Sample} = 0.1121$. The standard reagent's concentration is 4.3mM.

$$LDL - C\ mM = \frac{(A_{2,Sample} - A_{1,Sample}) - (A_{2,Blank} - A_{1,Blank})}{(A_{2,Standard} - A_{1,Standard}) - (A_{2,Blank} - A_{1,Blank})} \times C_{Standard}$$
$$= \frac{(0.1121 - 0.0605) - (0.0576 - 0.0533)}{(0.5418 - 0.0842) - (0.0576 - 0.0533)} \times 4.3mM = 0.4487mM$$

2. Tissue or Cell Sample

For Microplate Reader:

$$LDL - C\ mmol/g = \frac{(A_{2,Sample} - A_{1,Sample}) - (A_{2,Blank} - A_{1,Blank})}{(A_{2,Standard} - A_{1,Standard}) - (A_{2,Blank} - A_{1,Blank})} \times C_{Standard} \div C(mgprot/g)$$

For Automatic Biochemical Analyzer:

$$LDL - C\ mmol/g = \frac{A_{2,Sample-}A_{1,sample}}{A_{2,Standard} - A_{1,Standard}} \times C_{Standard}\ (4.3mM) \div C(mgprot/g)$$

Example: 2.5μL homogenate from mouse's liver was measured and absorbance values are listed below:

$A_{1,Blank} = 0.0361, A_{2,Blank} = 0.0432,\ A_{1,Standard} = 0.0658,\ A_{2,Standard} = 0.1297,$ $A_{1,Sample} = 0.1255\ and\ A_{2,Sample} = 0.1671$. The standard reagent's concentration is 4.3mM. Also, the protein concentration is measured to be 12.0121 g/L.

$$LDL - C\ mmol/g = \frac{(A_{2,Sample} - A_{1,Sample}) - (A_{2,Blank} - A_{1,Blank})}{(A_{2,Standard} - A_{1,Standard}) - (A_{2,Blank} - A_{1,Blank})} \times C_{Standard} \div C(mgprot/mg)$$
$$= \frac{(0.1671 - 0.1255) - (0.0432 - 0.0361)}{(0.1297 - 0.0658) - (0.0432 - 0.0361)} \times 4.3 \div 12.0121 = 0.0910mmol/g$$

5. Performance Index

1. Blank tube absorbance value≤0.050.
2. Sensitivity: The absorbance value difference ΔA is 0.180-0.280 for the 2.60mmol/L standard solution.
3. Restriction: Concentration range permitted is 2-12mM with r^2>0.995.
4. Accuracy: The coefficient of variation≤8% and relative deviation among batches ≤10%
5. Stability: Reagent in the original package should be preserved at 2-8 ℃ without light-struck and is stable within 12 months. Unsealed packages can be preserved for one month under same condition.

6. Notes

1. This assay kit is designed strictly for scientific 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. Reagents 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.