PRODUCT INFORMATION & MANUAL

High-density Lipoprotein Cholesterol/HDL-C Assay Kit (Colorimetric)

NBP3-25824

For research use only.
Not for diagnostic or therapeutic procedures.

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（**FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !**）

High-Density Lipoprotein Cholesterol (HDL-C) Colorimetric Assay Kit (Double Reagents)

Catalog No: NBP3-25824
Method: Colorimetric method
Specification: 100Assays **(**Can detect 96 samples without duplication)
Measuring instrument: Spectrophotometer
Detection range: 0.065-3.8 mmol/L

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help.

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Application

This kit can be used for detection of high-density lipoprotein cholesterol (HDL-C) content in serum, plasma, cells, culture supernatant and tissue samples.

Detection principle

The generated red purple pigment have a maximum absorption peak at 546 nm. Measure the OD value at 600 nm and the HDL-C content in the sample can be calculated.

Kit components

Reagent	Specification	Components	Concentration	Storage
Reagent 1	75 mL × 1 vial	Good's Buffer	50 mmol/L	2～8℃ Shading light
		Toos	1 mmol/L	
		$MgCl_2$ $6H_2O$	15 mmol/L	
		Cholesterol oxidase	≥ 3 KU	
		Peroxidase (POD)	≥ 5 KU	
Reagent 2	25 mL × 1 vial	Good's Buffer	50 mmol/L	
		4-ampyrone	0.2 mmol/L	
		$MgCl_2$ $6H_2O$	15 mmol/L	
		Cholesterol esterase	≥ 3 KU	
		Surfactant	0.1%	
Standard	Powder × 1 vial	Cholesterol	1.05 mmol/L	

The preparation of the standard: dissolve a vial of standard powder with 200 μL double distilled water before use.

Experimental instrument

Test tube, Micropipettor, Vortex mixer, Water bath, Spectrophotometer (600 nm)

Sample pretreatment

1. **Serum (Plasma):** Detect the sample directly. If the concentration is beyond the linear range, then dilute the sample with normal saline before detection.
2. **Culture supernatant sample:** Collect the culture medium, centrifuge at 1000 rpm for 10 min, and take the supernatant for detection.
   [Note]: It is generally recommended that the cell density should be more than 1×10^6 /mL.
3. **Tissue sample:** Accurately weigh the tissue weight, add 9 times the volume of homogenate media according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 2500 rpm for 10 min, then take the supernatant for detection.
   [Note]: (1) If the tissue sample is not a high-fat sample, the homogenate media should be phosphate buffer (0.1 mol/L, pH 7.4) or normal saline.
   (2) If the tissue sample is high-fat sample or partly high lipid sample, the homogenate media should be absolute alcohol.
4. **Cell sample:**
   Cell collection: Take the prepared cell suspension and centrifuge at 1000 rpm for 10 min. Discard the supernatant and keep the cell sediment. Wash the sediment with iso-osmia buffer (0.1 mol/L, pH7~7.4 phosphate buffer was recommended) 1~2 times, centrifuge at 1000 rpm for 10 min. Discard the supernatant and keep the cell sediment.
   Cell disruption: Add 0.2~0.3 mL of homogenate media (0.1 mol/L, pH7~7.4 phosphate buffer or normal saline was recommended). Sonicate in ice water bath (power: 300 W, 3~5 second/time, interval for 30 sec, repeat for 3~5 times) or grind with hand-operated. The prepared homogenate kept for detection without centrifugation. The cell can also be lysed with the cell lysate buffer (Triton X-100, 1~2%, 30~40 min), then take the prepared lysate for detection directly without centrifugation.
   [Note]: It is generally recommended that the cell density should be more than 1×10^6/ml. The disrupted cell can be observed with microscope that whether the cell is broken completely.

Operation steps

Operate with test tubes. Colorimetric assay by spectrophotometer			
	Blank well	Standard well	Sample well
Distilled water (μL)	10		
Standard (μL)		10	
Sample (μL)			10
Reagent 1 (μL)	750	750	750
Mix fully and incubate at 37℃ for 5 min. Set to zero with double distilled water and measure the OD value (A1) of each tube at 546 nm with spectrophotometer.			
Reagent 2 (μL)	250	250	250
Mix fully and incubate at 37℃ for 5 min. Set to zero with double distilled water and measure the OD value (A2) of each tube at 600 nm with spectrophotometer.			

Calculation of results

1. For of serum and other liquid sample:

$$\text{HDL} - \text{C Content}\ (mmol/L) = \frac{(\text{A2}_{\text{Sample}} - \text{A1}_{\text{Sample}}) - (\text{A2}_{\text{Blank}} - \text{A1}_{\text{Blank}})}{(\text{A2}_{\text{Standard}} - \text{A1}_{\text{Standard}}) - (\text{A2}_{\text{Blank}} - \text{A1}_{\text{Blank}})} \times \text{Concentration of standard}\ (mmol/L)$$

2. For tissue and cell sample:

$$\text{HDL} - \text{C Content}\ (mmol/gprot) = \frac{(\text{A2}_{\text{Sample}} - \text{A1}_{\text{Sample}}) - (\text{A2}_{\text{Blank}} - \text{A1}_{\text{Blank}})}{(\text{A2}_{\text{Standard}} - \text{A1}_{\text{Standard}}) - (\text{A2}_{\text{Blank}} - \text{A1}_{\text{Blank}})} \times \text{Concentration of standard}\ (mmol/L) \div \text{Protein concentration of tested sample}\ (gprot/L)$$

Performance index

1. The absorbance of blank tube is ≤ 0.010 (optical path = 0.5 cm).
2. **Linear range:** 0.065~3.8 mmol/L, $r^2 > 0.995$.
3. **Sensitivity:** The absorbance value of ΔA is between 0.087~0.153 when testing 1.3 mmol/L samples.
4. **Accuracy:** Relative deviation ≤ 10%.
5. **Precision:** intra-CV≤ 3%, inter-CV≤ 5%.
6. **Stability:** The validity of kit is 12 months when stored at 2-8℃ in the dark. It is stable for 1 months when stored at 2-8℃ in the dark after opening.

Notes

1. This product is for scientific research use only, not for clinical diagnosis.
2. The validity of kit is 12 months.
3. Do not use components from different batches of kit.
4. If the sample content is beyond the maximum limit, please dilute the sample with normal saline before detection, and multiply the result by the dilution ratio.
5. Protect the reagent from contamination of glucose, cholesterol, etc.
6. The amount of reagent and sample can be increased and decreased proportionately according to the volume of cuvette.