PRODUCT INFORMATION & MANUAL

Low-density Lipoprotein Cholesterol/LDL-C Assay Kit (Colorimetric)

NBP3-25882

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

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Novus kits are guaranteed for 6 months from date of receipt

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

Low-Density Lipoprotein Cholesterol (LDL-C) Colorimetric Assay Kit (Double Reagents)

Catalog No: NBP3-25882
Method: Colorimetric method
Specification: 100Assays **(**Can detect 96 samples without duplication)
Measuring instrument: Spectrophotometer
Detection range: 0.2-12 mmol/L

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

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

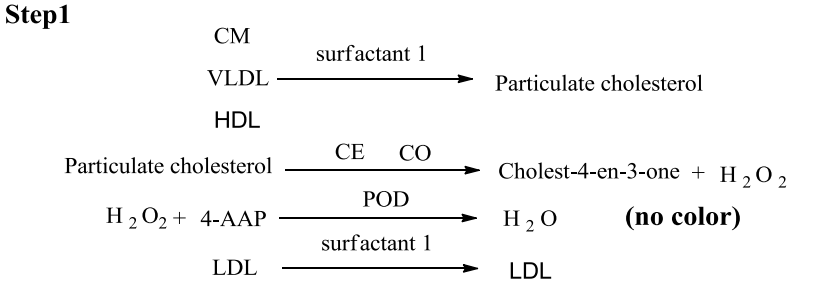
Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Application

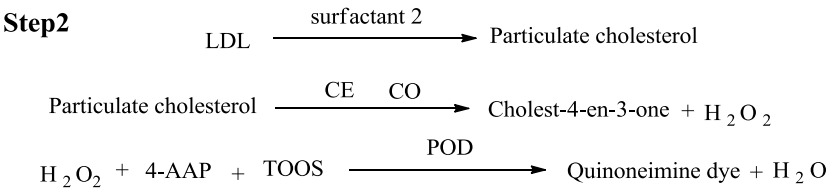
This kit can be used for detection of low-density lipoprotein cholesterol (LDL-C) content in serum, plasma, cells, culture supernatant and tissue samples.

Detection principle

Step1



Step2



The coloured substance have a maximum absorption peak at 546 nm. Measure the OD value at 546 nm and the LDL-C content in the sample can be calculated.

Kits components

Reagent	Component	Specification	Storage
Reagent 1	Enzyme Working Solution 1	75 mL ×1 vial	2-8℃, shading light
Reagent 2	Enzyme Working Solution 2	25 mL ×1 vial	2-8℃, shading light
Reagent 3	Cholesterol Standard	Powder ×1 vial	2-8℃, shading light
The preparation of 2.4 mmol/L the standard: dissolve a vial of standard powder with 200 μL double distilled water before use.			

Experimental instrument

Test tube, Micropipettor, Vortex mixer, 37℃ water bath/gas bath, Spectrophotometer (546nm)

Sample preparation

1. **Serum (Plasma):** Detect the sample directly. If the concentration is beyond the linear range, then dilute the sample with saline before detection.

2. **Culture fluid sample:** Centrifuge it at 1000 rpm for 10 min, take the supernatant for test.
   [Note]: It is generally recommended that the cell density should be more than 1 millon/mL.

3. **Tissue sample:** Accurately weigh the tissue weight, add 9 times the volume of homogenization medium (0.1mol/L, pH7-7.4 phosphate buffer or normal saline) 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 there are no high lipid samples exist in the tissue sample, the homogenate media should be extracted with phosphate buffer (0.1 mol/L, pH 7.4) or saline.

   (2) If the tissue sample is high lipid sample or partly high lipid sample, the homogenate media should be extracted with absolute alcohol.

4. **Cell sample:**

   (1) Cell collection: Take the prepared cell suspension and centrifuge at 1000 rpm for 10 min. Remove 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 1000rpm for 10min and then remove the supernatant and keep the cell sediment.

   (2) Cell disruption: Add 0.2~0.3 mL of homogenate media (0.1 mol/L, pH7~7.4 phosphate buffer or saline was recommended). Sonicate in ice water bath (power: 300W, 3~5 second/time, interval for 30 sec, repeat for 3~5 times) or grind with hand-operated. The prepared homogenate liquid kept for detection without centrifugation. The cell can also be lysed with the cell lysate buffer (Triton X-100, 1~2%, lysate for 30~40 min), then take the prepared homogenate liquid for detection directly without centrifugation.

   [Note]:

   It is generally recommended that the cell density should be more than 1millon/ml. The disrupted cell can be observed with microscope to check that whether the cell is broken completely.

Operation steps

Operate with test tubes. Colorimetric assay with Spectrophotometer.			
	Blank tube	Standard tube	Sample tube
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 the spectrophotometer to zero with distilled water and measure the absorbance value (A1) of each tube at 546 nm wavelength with 0.5 cm optical path cuvette.			
Reagent 2 (μL)	250	250	250
Mix fully and incubate at 37℃ for 5 min. Set the spectrophotometer to zero with distilled water and measure the absorbance value (A2) of each tube at 546 nm wavelength with 0.5 cm optical path cuvette.			

Calculation of results

1. **Calculation formula of serum and other liquid sample:**

   LDL-C content (*mmol/L*)

   $$= \frac{(A2_{Sample} - A1_{Sample}) - (A2_{Blank} - A1_{Blank})}{(A2_{Standard} - A1_{Standard}) - (A2_{Blank} - A1_{Blank})} \times \text{Concentration of standard}(mmol/L)$$

2. **Calculation formula of tissue and cell sample:**

   LDL-C content (*mmol/gprot*)

   $$= \frac{(A2_{Sample} - A1_{Sample}) - (A2_{Blank} - A1_{Blank})}{(A2_{Standard} - A1_{Standard}) - (A2_{Blank} - A1_{Blank})}$$

   $$\times \text{Concentration of standard } (mmol/L) \div \text{ Protein concentration of sample } (gprot/L)$$

Performance index

1. The absorbance of the blank tube is ≤ 0.050 (Optical path is 0.5 cm).
2. **Sensitivity:** The absorbance value ΔA is between 0.180~0.280 when testing 2.6 mmol/L samples.
3. **Linear range:** 0.2~12 mmol/L, $r^2 > 0.995$.
4. **Precision:** intra CV ≤ 8%, inter CV ≤ 10%.
5. **Stability:** This kit can be stored at 2~8℃ with shading light for 12 months and can be stable for 1 month since it is opened with the same storage condition.

Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 12 months.
4. Do not use components from different batches of kit.
5. If the sample content is beyond the maximum limit, please dilute the sample with saline before detection, and multiply the result by the dilution ratio.
6. Protect the reagent from contamination of glucose, cholesterol, etc.
7. The amount of reagent and sample can be increased and decreased according to the volume of cuvette.