

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

**Catalog No: E-BC-F034**

**Specification: 48T(32 samples)/96T(80 samples)**

**Measuring instrument: Fluorescence Microplate reader**

**(Ex/Em=535/587 nm)**

**Detection range: 2.52-260  $\mu$ mol/L**

## **Elabscience<sup>®</sup> HDL, LDL/VLDL and TC Fluorometric Assay Kit**

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:

Toll-free: 1-888-852-8623

Tell: 1-832-243-6086

Fax: 1-832-243-6017

Email: [techsupport@elabscience.com](mailto:techsupport@elabscience.com)

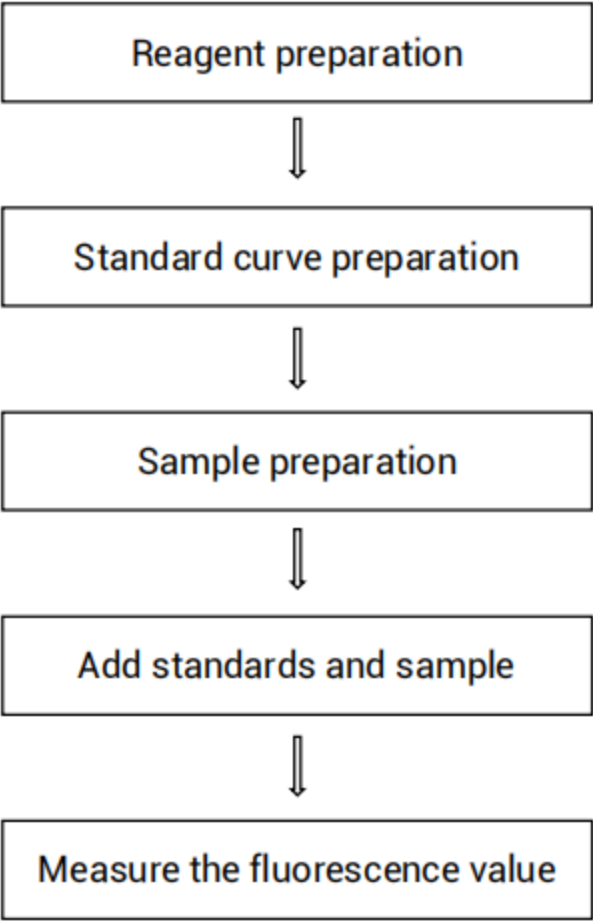
Website: [www.elabscience.com](http://www.elabscience.com)

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

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**Assay summary**



## **Intended use**

This kit can be used to detect the content of high density lipoprotein (HDL), low density lipoprotein (LDL)/very low density lipoprotein (VLDL) and total cholesterol (TC) in serum, plasma, animal tissue and cell samples.

## **Detection principle**

High density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) are the core indicators for evaluating blood lipid status. High LDL levels and low HDL levels are closely associated with increased cardiovascular disease risk. HDL is known as "good cholesterol" with anti-atherosclerotic effects; LDL is known as "bad cholesterol", and excess LDL tends to deposit on blood vessel walls to form plaques.

This kit uses a selective separation reagent to separate HDL from LDL/VLDL. The separated cholesterol is catalyzed by cholesterol esterase and cholesterol oxidase to produce 4-cholesten-3-one and hydrogen peroxide. In the presence of peroxidase (POD), hydrogen peroxide reacts with a non-fluorescent probe to generate a strong red fluorescent resorufin analog. The fluorescence intensity is measured at an excitation wavelength of 535 nm and an emission wavelength of 587 nm, which is proportional to the cholesterol concentration. HDL and LDL/VLDL contents can be calculated separately. Total cholesterol (TC) can be directly measured without separation.

## Kit components & storage

| Item      | Component                    | Size 1(48 T)     | Size 2(96 T)    | Storage                         |
|-----------|------------------------------|------------------|-----------------|---------------------------------|
| Reagent 1 | Separation Solution          | 11 mL × 1 vial   | 22 mL × 1 vial  | 2-8°C, 12 months, shading light |
| Reagent 2 | Reaction Solution            | 8.5 mL × 1 vial  | 17 mL × 1 vial  | 2-8°C, 12 months, shading light |
| Reagent 3 | Chromogenic Agent            | 0.35 mL × 1 vial | 0.7 mL × 1 vial | 2-8°C, 12 months, shading light |
| Reagent 4 | 5.9 mmol/L Standard Solution | 0.2 mL × 1 vial  | 0.4 mL × 1 vial | 2-8°C, 12 months                |
|           | Black Microplate             | 96 wells         |                 | No requirement                  |
|           | Plate Sealer                 | 2 pieces         |                 |                                 |
|           | Sample Layout Sheet          | 1 piece          |                 |                                 |

Note: All the reagents should be stored according to the table. The reagents from different kits can not be mixed or used interchangeably. For liquid reagents with small volumes or powders, centrifuge them before use to prevent loss.

## Instruments

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Incubator (37°C)

## Materials required but not provided

Double distilled water, 1×PBS (0.01 M, pH 7.4), Isopropanol (AR)

## Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② The preparation of Working Solution:

Before testing, please prepare sufficient Substrate Working Solution according to the test wells. For example, prepare 1040  $\mu\text{L}$  of Working Solution (mix well 1000  $\mu\text{L}$  of Reaction Solution and 40  $\mu\text{L}$  of Chromogenic Agent). The substrate working solution should be prepared on spot protected from light and used up within 8 hours.

③ Pre-incubate the Working Solution at 37°C for 15 min before use.

④ The preparation of 295  $\mu\text{mol/L}$  Standard Solution:

Before testing, please prepare sufficient 295  $\mu\text{mol/L}$  Standard Solution according to the test wells. For example, prepare 1000  $\mu\text{L}$  of 295  $\mu\text{mol/L}$  Standard Solution (mix well 950  $\mu\text{L}$  of Isopropanol (AR) and 50  $\mu\text{L}$  of 5.9 mmol/L Standard Solution). The 295  $\mu\text{mol/L}$  Standard Solution can be stored at 2~8°C for 7 days.

⑤ Standard curve preparation:

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 295  $\mu\text{mol/L}$  Standard Solution with Isopropanol (AR) diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 59, 118, 147.5, 177, 206.5, 236, 295  $\mu\text{mol/L}$ . Reference is as follows:

| Item                                                                        | ①        | ②         | ③          | ④            | ⑤          | ⑥            | ⑦          | ⑧          |
|-----------------------------------------------------------------------------|----------|-----------|------------|--------------|------------|--------------|------------|------------|
| <b>Concentration (<math>\mu\text{mol/L}</math>)</b>                         | <b>0</b> | <b>59</b> | <b>118</b> | <b>147.5</b> | <b>177</b> | <b>206.5</b> | <b>236</b> | <b>295</b> |
| <b>295 <math>\mu\text{mol/L}</math> Standard (<math>\mu\text{L}</math>)</b> | 0        | 40        | 80         | 100          | 120        | 140          | 160        | 200        |
| <b>Isopropanol(<math>\mu\text{L}</math>)</b>                                | 200      | 160       | 120        | 100          | 80         | 60           | 40         | 0          |

## Sample preparation

**Serum and plasma:** detect directly. If not detected on the same day, the serum or plasma can be stored at  $-80^{\circ}\text{C}$  for a month.

### Tissue sample:

- ① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).
- ② Wash tissue in cold PBS (0.01 M, pH 7.4).
- ③ Homogenize 20 mg tissue in 180  $\mu\text{L}$  Isopropanol (AR) with a dounce homogenizer at  $4^{\circ}\text{C}$ .
- ④ Centrifuge at  $10000\times g$  for 10 min at  $4^{\circ}\text{C}$  to remove insoluble material. Collect supernatant and keep it on ice for detection.

### Cell sample

- ① Harvest the number of cells needed for each assay (initial recommendation  $1\times 10^6$  cells).
- ② Wash cells with PBS (0.01 M, pH 7.4).
- ③ Homogenize  $1\times 10^6$  cells in 200  $\mu\text{L}$  Isopropanol (AR) , and sonicate for 10 min using an ultrasonic cell disruptor.
- ④ Centrifuge at  $10000\times g$  for 10 min at  $4^{\circ}\text{C}$  to remove insoluble material. Collect supernatant and keep it on ice for detection.

### Dilution of sample

For TC assay, all samples shall be diluted 20-fold.

For HDL and LDL assays, The recommended dilution factors for sample solutions after separation are as follows(for reference only):

| Sample type   | Dilution factor |
|---------------|-----------------|
| Human serum   | 2-3             |
| Mouse serum   | 2-3             |
| Rat serum     | 2-3             |
| Porcine serum | 1               |

|                                         |     |
|-----------------------------------------|-----|
| Guinea pig serum                        | 1   |
| Horse serum                             | 1   |
| 10% Porcine liver tissue homogenization | 2-3 |
| 10% Mouse liver tissue homogenization   | 1   |
| 10% Mouse kidney tissue homogenization  | 1   |
| 10% Mouse spleen tissue homogenization  | 1   |
| 10% Mouse heart tissue homogenization   | 1   |
| 10% Mouse muscle tissue homogenization  | 1   |
| $1 \times 10^6$ Hela cells              | 1   |
| $1 \times 10^6$ 293T cells              | 1   |

Note: Samples should be separated before dilution. The diluent for serum samples is PBS (0.01 M, pH=7.4); the diluent for tissue and cell samples is Isopropanol (AR).

## Operating steps

HDL and LDL/VLDL separation:

Take 0.2 mL of sample solution into a 1.5 mL EP tube, add 0.2 mL of Reagent 1, mix well and place on ice for 30 min. After standing, centrifuge at 12000×g for 15 min at 4°C. Take 0.2 mL of supernatant into a new 1.5 mL EP tube and label as HDL. Carefully remove the remaining supernatant, add 0.2 mL of PBS (0.01 mol/L, pH=7.4) to the precipitate, and pipette repeatedly until the precipitate is completely redissolved, label as LDL/VLDL.

- ① Pre-incubate the Working Solution at 37°C for 15 min.
- ② Standard well: Add 10  $\mu$ L of Standard Solution with different concentrations to the corresponding wells.  
Sample well: Add 10  $\mu$ L of sample to the corresponding wells.
- ③ Add 150  $\mu$ L of Working Solution to standard and sample wells.
- ④ Shake the plate for 3 s, incubate at 37°C for 5 min. Measure the fluorescence value of each well at Ex/Em=535/587 nm.

## Calculation

### The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean fluorescence value of the blank (Standard #①) from all standard readings. This is the Corrected fluorescence value.
3. Plot the standard curve by using Corrected fluorescence value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve ( $y = ax + b$ ) with graph software (or EXCEL).

### The sample:

#### Tissue sample:

$$\text{HDL content} \\ (\mu\text{mol/kg wet weight}) = (\Delta F - b) \div a \div (m \div V) \times f \times 2^*$$

$$\text{LDL/VLDL content} \\ (\mu\text{mol/kg wet weight}) = (\Delta F - b) \div a \div (m \div V) \times f$$

$$\text{TC content} \\ (\mu\text{mol/kg wet weight}) = (\Delta F - b) \div a \div (m \div V) \times f$$

#### Serum and plasma samples:

$$\text{HDL content} \\ (\mu\text{mol/L}) = (\Delta F - b) \div a \times f \times 2^*$$

$$\text{LDL/VLDL content} \\ (\mu\text{mol/L}) = (\Delta F - b) \div a \times f$$

$$\text{TC content} \\ (\mu\text{mol/L}) = (\Delta F - b) \div a \times f$$

#### Cell sample:

$$\text{HDL content} \\ (\text{nmol}/10^6) = (\Delta F - b) \div a \div (n \div V) \times f \times 2^*$$

$$\text{LDL/VLDL content} \frac{(\text{nmol}/10^6)}{(\text{nmol}/10^6)} = (\Delta F - b) \div a \div (n \div V) \times f$$

$$\text{TC content} \frac{(\mu\text{mol}/\text{L})}{(\mu\text{mol}/\text{L})} = (\Delta F - b) \div a \div (n \div V) \times f$$

### **[Note]**

$\Delta F$ : The absolute fluorescence value of sample,  $F_{\text{sample}} - F_{\text{blank}}$  ( $F_{\text{blank}}$  is the fluorescence value when the standard concentration is 0).

$m$ : The weight of tissue, g.

$V$ : The volume of Isopropanol (AR) in the preparation step of sample, mL.

$n$ : The number of cell sample/ $10^6$ .

$f$ : Dilution factor of sample before tested.

2\*:The sample was mixed with the separation buffer at a 1:1 (v/v) ratio, resulting in a 2-fold dilution of HDL in the supernatant. The measured HDL concentration was multiplied by a dilution factor of 2.

## Appendix I Performance Characteristics

### 1. Parameter:

#### Intra-assay Precision

Three rat serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

| Parameters                 | Sample 1 | Sample 2 | Sample 3 |
|----------------------------|----------|----------|----------|
| Mean ( $\mu\text{mol/L}$ ) | 876.96   | 698.88   | 1071.35  |
| %CV                        | 5.1      | 5.4      | 4.3      |

#### Inter-assay Precision

Three rat serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

| Parameters                 | Sample 1 | Sample 2 | Sample 3 |
|----------------------------|----------|----------|----------|
| Mean ( $\mu\text{mol/L}$ ) | 876.96   | 698.88   | 1071.35  |
| %CV                        | 9.3      | 7.8      | 3.1      |

#### Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 98%.

|                          | Sample 1 | Sample 2 | Sample 3 |
|--------------------------|----------|----------|----------|
| Expected Conc. (U/gprot) | 44.25    | 88.5     | 118      |
| Observed Conc. (U/gprot) | 42.12    | 87.49    | 118.30   |
| Recovery rate (%)        | 95       | 99       | 100      |

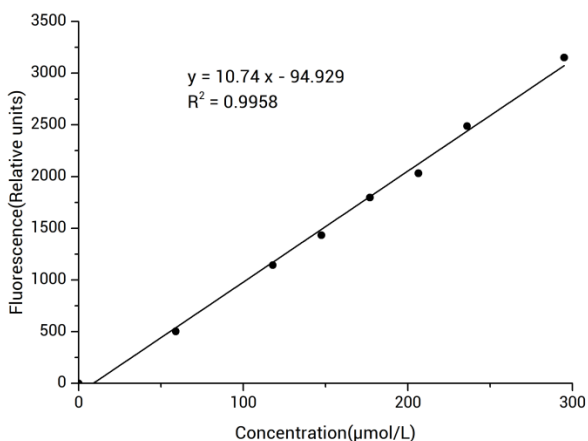
#### Sensitivity

The analytical sensitivity of the assay is  $2.52 \mu\text{mol/L}$ . This was determined by adding two standard deviations to the mean fluorescence value when the zero standard was assayed 20 times, and calculating the corresponding concentration.

## 2. Standard curve:

As the fluorescence value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

| Concentration<br>( $\mu\text{mol/L}$ ) | 0   | 59   | 118  | 147.5 | 177  | 206.5 | 236  | 295  |
|----------------------------------------|-----|------|------|-------|------|-------|------|------|
| Fluorescence value                     | 844 | 1331 | 2011 | 2254  | 2780 | 2789  | 3346 | 3818 |
|                                        | 857 | 1376 | 1978 | 2313  | 2516 | 2975  | 3331 | 4184 |
| Average<br>fluorescence value          | 851 | 1354 | 1995 | 2284  | 2648 | 2882  | 3339 | 4001 |
| Corrected<br>fluorescence value        | 0   | 503  | 1144 | 1433  | 1798 | 2032  | 2488 | 3151 |



## Appendix II Example Analysis

### Example analysis:

Take 10  $\mu\text{L}$  of 3-fold diluted mouse serum and carry the assay according to the operation steps. The results are as follows:

standard curve:  $y = 10.74x - 94.929$ . the average fluorescence value of HDL sample well is 4621, the average fluorescence value of LDL sample well is 2938, the average fluorescence value of the blank well is 851, the calculation result is:

$$\text{HDL content } (\mu\text{mol/L}) = (4621 - 851 + 94.929) \div 10.74 \times 3 \times 2 = 2159 \mu\text{mol/L}$$

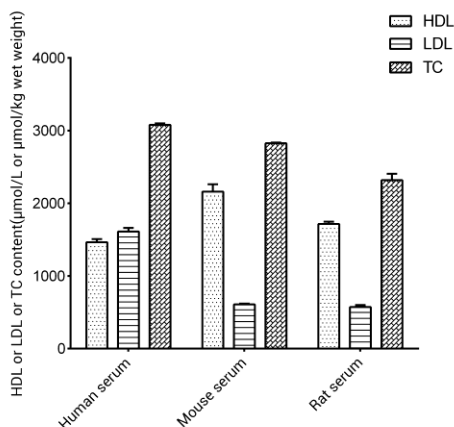
$$\text{LDL/VLDL content } (\mu\text{mol/L}) = (2938 - 851 + 94.929) \div 10.74 \times = 609.5 \mu\text{mol/L}$$

Take 10  $\mu\text{L}$  of 20-fold diluted mouse serum and carry the assay according to the operation steps. The results are as follows:

standard curve:  $y = 10.74x - 94.929$ . the average fluorescence value of TC sample well is 2274, the average fluorescence value of the blank well is 851, the calculation result is:

$$\text{TC content } (\mu\text{mol/L}) = (2274 - 851 + 94.929) \div 10.74 \times 20 = 2826 \mu\text{mol/L}$$

Detect human serum, (dilute for 2 times), mouse serum (dilute for 3 times), and rat serum (dilute for 2 times), according to the protocol, the result is as follows:



## **Statement**

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.

