Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

EZScreen™ Total Cholesterol & Cholesteryl Ester Colorimetric Assay Kit (384-Well)

10/16

(Catalog # K957-400; 400 assays; Store at -20°C)

I. Introduction:

The EZScreen™ Cholesterol and Cholesteryl Ester Colorimetric assay Kit provides a simple method for sensitive quantification of free cholesterol, cholesteryl esters, or both by a colorimetric method in a high throughput format. The majority of the cholesterol in blood is in the form of cholesteryl esters which can be hydrolyzed to cholesterol by cholesterol esterase. The cholesterol is then oxidized yielding H_2O_2 which reacts with a sensitive cholesterol probe to produce a signal (OD 570 nm). The EZScreen™ assay detects total cholesterol (cholesterol and cholesteryl esters) in the presence of cholesterol esterase or free cholesterol in the absence of cholesterol esterase. The cholesteryl ester content can be determined by subtracting the value of free cholesterol from the total (cholesterol + cholesteryl esters). The assay is quantitative, rapid, simple, and sensitive. The 384-well format allows for the screening of a large number of samples on a single plate in high throughput screening mode. The kit can detect 0.25 to 1.25 µg/well of cholesterol in various biological samples.

II. Applications:

- Measurement of cholesterol in various biological samples (tissues/cells)
- Analysis of lipid metabolism in various cells

III. Sample Type:

- Serum, plasma, & other body fluids
- Growth media
- Cultured cells: Adherent or suspension cells

IV. Kit Contents:

Components	K957-400	Cap Code	Part Number
Cholesterol Assay Buffer	25 ml	WM	K957-400-1
Cholesterol Probe (in DMSO, anhydrous)	0.2 ml	Red	K957-400-2A
Enzyme Mix (lyophilized)	1 vial	Green	K957-400-3
Cholesterol Esterase (lyophilized)	1 vial	Blue	K957-400-4
Cholesterol Standard (2 µg/µl)	100 µl	Yellow	K957-400-5

V. User Supplied Reagents and Equipment:

- 384-well clear plate with flat bottom
- Multi-well spectrophotometer with 384-well plate reading capability

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay. Keep enzymes and cholesterol standard on ice while using.

- **Cholesterol Assay Buffer**: Warm to room temperature prior to use. Store at -20°C or 4°C.
- **Cholesterol Probe**: Ready to use as supplied. Warm to room temperature to thaw the probe solution prior to use. Store at -20°C, protect from light. Use within two months.
- **Enzyme Mix**: Dissolve in 220 µl Cholesterol Assay Buffer. Aliquot & store at -20°C. Keep on ice while in use. Use within two months.
- **Cholesterol Esterase**: Dissolve in 220 µl Cholesterol Assay Buffer. Aliquot & store at -20°C. Keep on ice while in use. Use within two months.
- **Cholesterol Standard**: Keep on ice while in use.

VII. Cholesterol Assay Protocol:

1. **Sample Preparation:** Serum samples (0.5-10 µl/assay) should be diluted 10-fold in the Cholesterol Assay Buffer. Use 1-10 µl of diluted sample per well. Adjust the volume to 13.0 µl with Cholesterol assay buffer.

   Notes:

   a. For unknown samples, we suggest performing a pilot experiment & testing different sample dilutions with the Assay Buffer to ensure the readings are within the Standard Curve range.

   b. For samples having background, prepare parallel background well(s) containing the same amount of sample as in the test well.

   c. Instrument reader settings need to be adjusted according to the chosen 384-well plate. (The dimensions of the used 384-well plate may be available in the manual provided by the plate-manufacturer).

2. **Cholesterol Standard Curve:** Dilute the Cholesterol Standard to 125 ng/µl by adding 10 µl of the Cholesterol Standard to 150 µl of Cholesterol Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells on a 384-well plate to generate 0, 250, 500, 750, 1000, and 1250 ng/well of Cholesterol Standard. Adjust volume to 13.0 µl/well with Cholesterol Assay Buffer.

3. **Cholesterol Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 12.0 µl Mix containing:

	Standard/Total Cholesterol	Free Cholesterol[a]	*Background Control Mix
Cholesterol Assay Buffer	10.5 µl	11.0 µl	11.0 µl
Cholesterol Probe	0.5 µl	0.5 µl	0.5 µl
Cholesterol Enzyme Mix	0.5 µl	0.5 µl	----
Cholesterol Esterase[a,b]	0.5 µl	----	0.5 µl

Mix and add 12.0 µl of the Reaction Mix to each well containing the Standard, or test samples. Mix well.
* For samples having background, add 12.0 µl of the background control mix to the sample background control well(s).

Notes:

a. Cholesterol esterase hydrolyzes cholesteryl ester to cholesterol. Cholesterol esterase is used to detect both free cholesterol and cholesteryl esters. For detecting free cholesterol only: prepare a reagent mix containing the enzyme mix only, as indicated above. For detecting Cholesterol esters only: subtract the value of free cholesterol from the value of the total cholesterol (cholesterol and cholesteryl esters).

b. The Cholesterol Standard contains a mixture of free cholesterol and cholesteryl esters in a ratio similar to that of serum. Cholesterol Esterase must be added to the standard curve reaction to convert all to cholesterol.

4. **Measurement:** Incubate the reaction for 60 min. at 37°C, protect from light. Measure absorbance (OD: 570 nm) in a microplate reader.

5. **Calculation:** Subtract 0 Standard reading from all readings which will be the corrected absorbance readings. If the sample background control reading is significant then subtract the sample background control reading from the sample reading. Plot the Cholesterol Standard Curve (OD: 570 nm vs ng Standard). Apply the corrected absorbance of the sample to the Cholesterol Standard Curve to get B ng of Cholesterol in the sample well.

Sample Cholesterol concentration (C) = B/V x D (ng/µl)

Where: **B** = is the amount of Cholesterol in the sample well from Standard Curve
V = sample volume added into the reaction well (µl).
D = sample dilution Factor

Cholesterol molecular weight: 386.15 g/mol. 1000 ng/ µl = 1 µg/ µl = 100 mg/dl

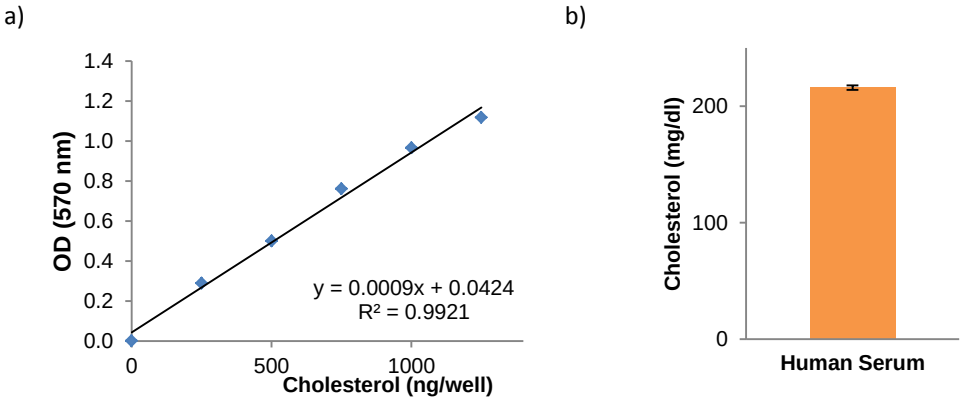

Figure: (a) Cholesterol Standard Curve; (b) Quantitation of Cholesterol in pooled human serum. 5 µl of 1:10 diluted Serum samples were added to the wells. Assays were performed according to the kit protocol. The calculated concentration of cholesterol: 216.2 ± 2 mg/dl.

VIII. RELATED PRODUCTS:

Free Fatty Acid Quantification Colorimetric/Fluorometric Kit (K612)
CETP Activity Fluorometric Assay Kit (K601)
HDL and LDL/VLDL Quantification Assay Kit (K613)
PicoProbe™ Triglyceride Quantification Kit (K614)
EZScreen™ Free Fatty Acid Colorimetric Assay Kit (K953)
EZScreen™ Triglyceride Assay Kit-384 Well Format (K952)
Triglyceride Quantification Colorimetric/Fluorometric Assay Kit (K622)
PLTP Activity Fluorometric Assay Kit (K604)
CETP Inhibitor Drug Screening Kit (Fluorometric) (K602)
PLTP Inhibitor Drug Screening Kit (Fluorometric) (K605)
Total Cholesterol and Cholesterol Ester Colorimetric Assay Kit (K603)
Total Cholesterol/ Cholesterol Ester Colorimetric Assay Kit II (K623)

FOR RESEARCH USE ONLY! Not to be used on humans.