May 14, 2019

UC Davis - Total Cholesterol (TC) Protocol

DOI
dx.doi.org/10.17504/protocols.io.ygeftte

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OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.ygeftte

External link: https://mmpc.org/shared/document.aspx?id=92&docType=Protocol

Protocol Citation: Peter Havel 2019. UC Davis - Total Cholesterol (TC) Protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.ygeftte

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Protocol status: Working
We use this protocol and it's working

Created: February 23, 2019

Last Modified: May 14, 2019

Protocol Integer ID: 20710

Keywords: Cholesterol

Abstract

Summary:

Cholesterol esters are enzymatically hydrolysed by cholesterol esterase to cholesterol and free fatty acids. Free cholesterol, including that originally present, is then oxidized by cholesterol oxidase to cholest-4-en-3one and hydrogen peroxide. The hydrogen peroxide combines with HBA and 4-aminoantipyrine to form a chromophore (quinoneimine dye) which may be quantitated at 500-550nm.

Materials

MATERIALS

- Calibrator **Fisher Diagnostics Catalog #**TR43002
- Reagents **Fisher Diagnostics Catalog #**TR13421
- PBS
- Microplate
- Platereader

Note:

Thermo Fisher Scientific, RRID:SCR_008452

1 Add 5 µl of calibrator and sample to each well.

IMPORTANT: Make sure not to add any bubbles to the wells when dispensing reagents, this will interfere with reading in the platereader.

2 Add 300 µl of reagent to each well. Incubate at 37°C for 5 minutes. Read at 540 nm.

IMPORTANT: If samples are hemolyzed,pipet a blank well with 5 µl sample and 300 µl PBS

3 Subtract blank readings from final readings. The assay will be linear so the unknown samples can be calculated as (Sample Absorbance ÷ Calibrator Absorbance) × Calibrator Concentration.