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Working

# UC Davis - Total Cholesterol (TC) Protocol

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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.

EXTERNAL LINK

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

MATERIALS

| NAME | CATALOG # | VENDOR | CAS NUMBER | RRID |
|---|---|---|---|---|
| Calibrator | TR43002 | Fisher Diagnostics | | |
| Reagents | TR13421 | Fisher Diagnostics | | |
| PBS | | | | |
| Microplate | | | | |
| Platereader | | | | |

MATERIALS TEXT

**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.