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

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PROTOCOL integer ID:
 20710

🌐 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-3-one 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

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⊗ Calibrator Fisher Diagnostics Catalog #TR43002

⊗ Reagents Fisher Diagnostics Catalog #TR13421

⊗ PBS Contributed by users

⊗ Microplate Contributed by users

⊗ Platereader Contributed by users

Note:

Thermo Fisher Scientific, [RRID:SCR_008452](#)

Keywords: Cholesterol

- 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 $(\text{Sample Absorbance} \div \text{Calibrator Absorbance}) \times \text{Calibrator Concentration}$.