D3405 Total Cholesterol (TC) Protocol

Version: 1
Replaced by version
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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.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Calibrator	Fisher Diagnostics	TR43002
Reagents	Fisher Diagnostics	TR13421
PBS		
Microplate		
Platereader		

Protocol:

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 5µl sample and 300µl 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.

Reagent Preparation:

Reagent – ready to use
PBS – ready to use