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Working

UC Davis - Triglyceride Protocol

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ABSTRACT

Summary:

Triglycerides are enzymatically hydrolyzed by lipase to free fatty acids and glycerol. The glycerol is phosphorylated by adenosine triphosphate (ATP) with glycerol kinase (GK) to produce glycerol-3- phosphate and adenosine diphosphate. Glycerol-3-phosphate is oxidized by dihydroxyacetone phosphate (DAP) by glycerolphosphate oxidase producing hydrogen peroxide (H2O2). In a Trinder5 type color reaction catalyzed by peroxidase, the H2O2 reacts with 4-aminoantipyrine (4-AAP) and 3,5-dichloro-2- hydroxybenzene sulfonate (DHBS) to produce a red colored dye. The absorbance of this dye is proportional to the concentration of triglycerides present in the sample.

EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=91&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR
Calibrator	TR43002	Fisher Diagnostics
Reagents	TR22203	Fisher Diagnostics
PBS		
Microplate		
Platereader		

MATERIALS TEXT

Reagent Preparation:

PBS – ready to use
Reagent – reconstitute with distilled water to make a 2X solution

1 Reconstitute powdered reagent with only 25 ml of distilled water to make a 2X solution.

2 Add 3 µl of calibrator and sample to each well.

3 Add 150 µl of PBS to each well. Read at 540 nm.

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

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4 Add 150 µl of 2X reagent to each well. Incubate at 37°C for 5 minutes. Read at 540 nm.

5 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.

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