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UC Davis - Triglyceride Protocol

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

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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.

Materials

MATERIALS

- Calibrator **Fisher Diagnostics Catalog #**TR43002
- Reagents **Fisher Diagnostics Catalog #**TR22203
- PBS
- Microplate
- Platereader

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.*

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.