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UC Davis - Triglyceride Protocol

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OPEN ACCESS

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

Created: Mar 07, 2019

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PROTOCOL integer ID:
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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

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☒ Calibrator **Fisher Diagnostics Catalog #TR43002**

☒ Reagents **Fisher Diagnostics Catalog #TR22203**

☒ PBS **Contributed by users**

☒ Microplate **Contributed by users**

☒ Platereader **Contributed by users**

Reagent Preparation:

PBS – ready to use

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

Keywords: Triglyceride

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