Triglyceride Protocol

Version: 1
Replaced by version
Edited by: Peter Havel - UC Davis Metabolism and Endocrinology Core

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.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Calibrator	Fisher Diagnostics	TR43002
Reagents	Fisher Diagnostics	TR22203
PBS		
Microplate		
Platereader		

Protocol:

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.

Reagent Preparation:

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