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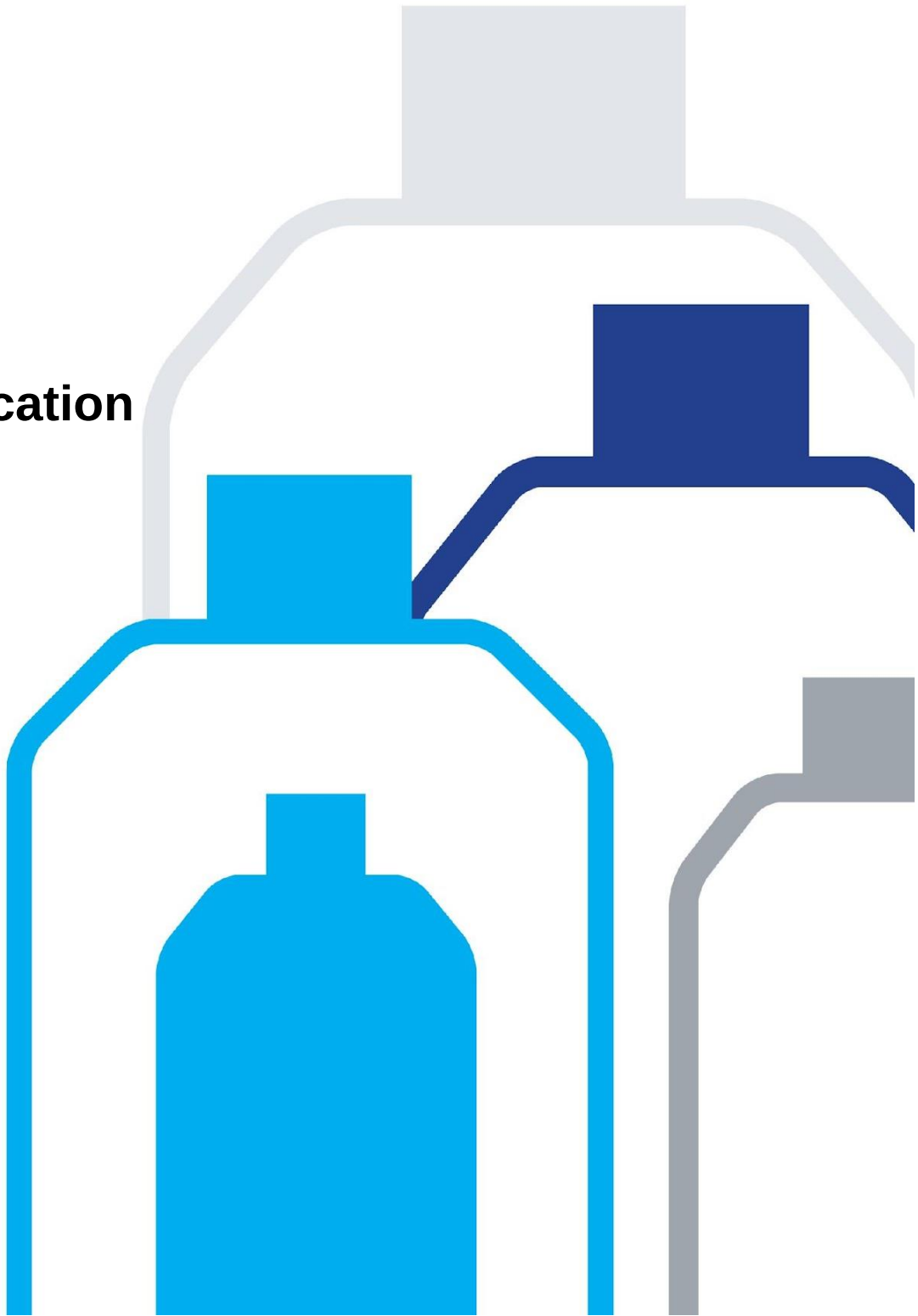
User Guide

LipoQ™

Lipid Quantification Assay

Cat LP01



Version 1.2

Contents

LipoQ™

Lipid Quantification Assay

Product Components

LP01-100 LipoQ™ Lipid Quantification Assay

- 1 x Lipid Quantification Reagent, 12 ml
- 1 x Lipid Standard, 1 mg
- 1 x User Guide

Storage

Store all components at 4°C. LipoQ™ has a shelf life of at least six months from receipt.

Please note that the lipid quantification reagent is light-sensitive and should be protected from light until use.

Equipment and materials required but not supplied with this kit

- Concentrated Sulfuric Acid (18M)
- Chloroform or another organic solvent
- 2 mL glass test tubes, 15 mL conical tubes, microcentrifuge tubes, or 96 well plates
- Single and multichannel micropipettes with disposable tips, and multichannel micropipette reservoir
- Thermal heat block

Introduction and assay principle

LipoQ™ uses the sulfo-phospo-vanillin method to measure the lipid content of a wide variety of sample types. Unsaturated fatty acids within a sample will react with concentrated sulfuric acid, which in combination with phospho-vanillin will form a pink-colored solution. The intensity of the pink colour formed is determined by the total lipid concentration within the sample, enabling a reliable colorimetric assay that can be read using a plate reader.

Each kit provides sufficient reagents to perform 100 tests, including standards.

Procedure

Please note: Sulfuric acid is highly corrosive and can damage certain types of plastics. Avoid using plastics that are sensitive to sulfuric acid, and test plastics prior to attempting this assay by adding 100 µL of 18M sulfuric acid and heating to 90ºC for 10 minutes. Sulfuric acid should be handled with care (see MSDS). Gloves, a lab coat, and protective eyewear should be worn during handling. Sulfuric acid should be stored in glassware only and pipetted in a fume hood.

A. Standard Preparation

1. Dissolve 1 mg Lipid Standard in 1 mL chloroform in a 2 mL test tube.
2. Evaporate chloroform at 60°C in a thermal heat block under a fume hood.
3. Add 250 µL deionised water to the dried Lipid Standard powder.
4. Ensure the cap is tightly fastened. Vortex intensively for 2 minutes at max speed.
5. Sonicate at 35 kHz at 45°C for 10 minutes.
6. Ensure the cap is tightly fastened. Vortex intensively for 2 minutes.
7. Prior to assay, form a dilution series of 16 µg – 0.25 µg. See table 1 below.

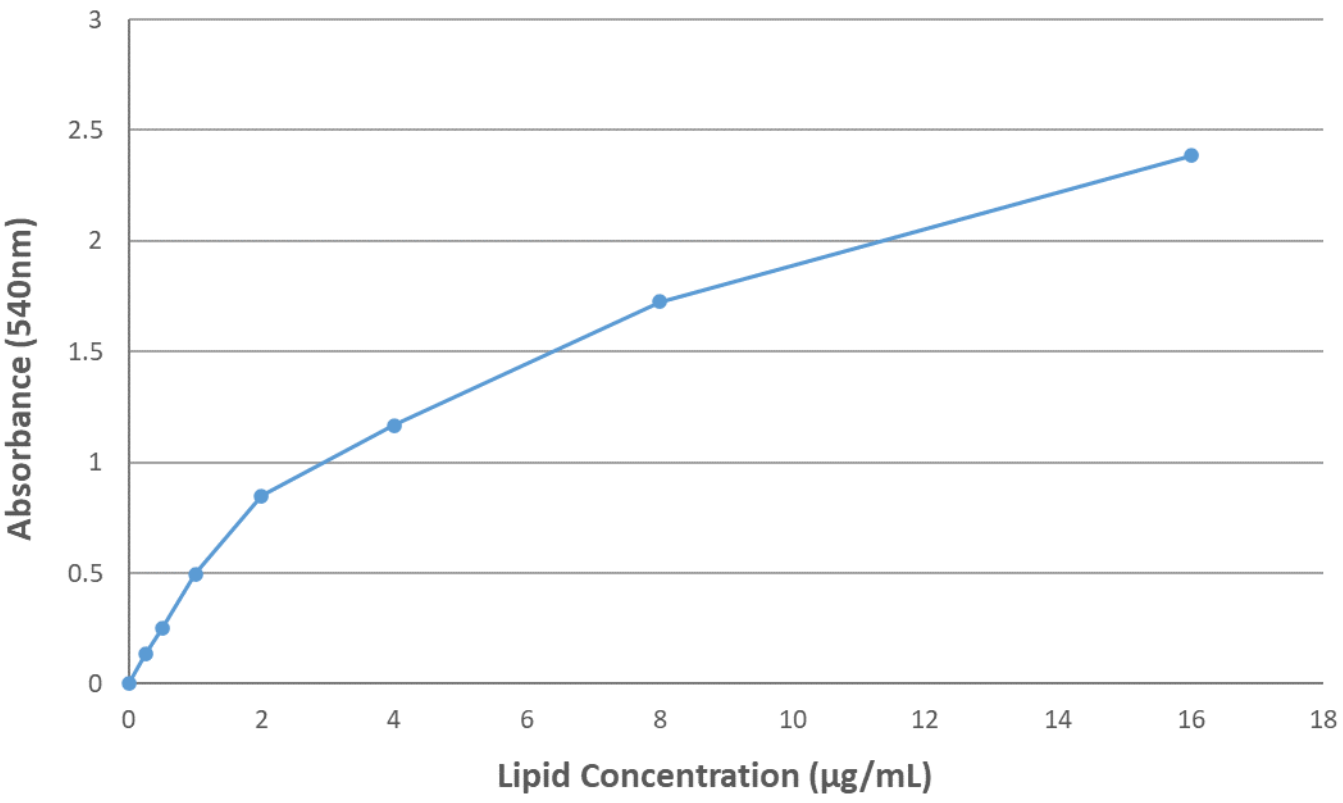
Lipid standard is stable at 4°C for at least three months once reconstituted. Intensive vortexing is required prior to the use of the lipid standard.

Tube number	Lipid standard (µL)	Diluted in PBS (µL)	Lipid standard concentration (500 µL solution)
1	1 mg (resuspended in 250 µL deionised water)	0	4 mg/ml
2	2 µL of Tube #1	498	16 µg/ml
3	250 µL of Tube #2	250	8 µg/ml
4	250 µL of Tube #3	250	4 µg/ml
5	250 µL of Tube #4	250	2 µg/ml
6	250 µL of Tube #5	250	1 µg/ml
7	250 µL of Tube #6	250	0.5 µg/ml
8	250 µL of Tube #7	250	0.25 µg/ml

Table 1. Lipid standard preparation.

B. Assay Procedure

1. Add 40 µL of sample, prepared in PBS, deionised water, or NaCl HEPES Buffer, or freshly prepared lipid standard serial dilution, in 1.5 mL Safe-Lock tubes.
2. Add 200 µL 18M sulfuric acid to the samples and standards.
3. Ensure the cap is tightly fastened. Vortex briefly.
4. In a fume hood, incubate open tubes at 90°C for 20 minutes.
5. Ensure the tube is tightly fastened. Cool to room temperature by placing at 4°C for at least 5 minutes.
6. Add 120 µL of the lipid quantification reagent to each tube.
7. Ensure the tube is tightly fastened. Vortex briefly.
8. Add 280 µL of each tube to a 96 well plate. 280 µL of PBS may be added to the 96 well plate as a negative control.
9. Incubate at 37°C for 1 hour.
10. Measure the absorbance at 540 nm.



Graph 1. Standard curve produced using LipoQ™ lipid quantification reagent upon the lipid standard supplied with the kit.

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