Technical Bulletin

Triglyceride Quantification Kit

Catalogue number MAK564

Product Description

Triglycerides (TG) are the main constituents of vegetable oil, animal fat, LDL, and VLDL, and play an important role as transporters of fatty acids as well as an energy source. TG is broken down into fatty acids and glycerol, after which both can serve as substrates for energy producing and metabolic pathways. High blood levels of TG are implicated in atherosclerosis, heart disease, and stroke as well as in pancreatitis[1].

The Triglyceride Quantification Kit provides a sensitive, easy assay to measure TG concentration in a variety of samples. In this assay, TG are converted to free fatty acids and glycerol. The glycerol is then oxidized to generate a colorimetric (570 nm)/fluorometric (λ_{ex} = 535 nm/λ_{em} = 587 nm) product. The kit is sensitive to detect ≥0.1 nmole (1 µM) fluorometrically or ≥0.4 nmole (4 µM) colorimetrically of triglycerides in various samples. The kit also detects monoglycerides and diglycerides. This kit is suitable for use with serum, plasma, milk and other biological fluids, and tissue and cell culture samples.

Components

The kit is sufficient for 100 colorimetric or fluorometric assays in 96-well plates.

- Triglyceride Assay Buffer 25 mL
  Catalogue Number MAK564A
- Triglyceride Probe, in DMSO 0.2 mL
  Catalogue Number MAK564B
- Lipase 1 vial
  Catalogue Number MAK564C
- Triglyceride Enzyme Mix 1 vial
  Catalogue Number MAK564D
- Triglyceride Standard (1 mM) 0.3 mL
  Catalogue Number MAK564E

Reagents and Equipment Required but Not Provided

- 96-well flat-bottom plate.
  - Clear plates for colorimetric assays (Catalogue number M2936 or equivalent)
  - Black plates with clear bottoms for fluorescence assays (Catalogue number CLS3631 or equivalent)
  - Cell culture or tissue culture treated plates are not recommended.
- Plate reader that is capable to read absorbance at wavelength of λ_{ex} = 535 nm/λ_{em} = 587 nm.
- Pipettors and Pipettes
- Vortex
- Tergitol™ (Catalogue number T1135 or equivalent)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or otheruses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on dry ice. Store components at -20 °C

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. Avoid repeated freeze/thaw cycles.

Triglyceride Assay Buffer (MAK564A)

Allow buffer to come to room temperature before use.

Triglyceride Probe (MAK564B)

Warm in 37 °C bath for 1–5 minutes to melt solution prior to use. Upon thawing, the probe is ready-to-use as supplied. Store at -20 °C, protected from light. Use within 2 months.

For the fluorescence assay, dilute an aliquot of the Triglyceride Probe Solution 5 to 10 fold with Assay Buffer, just prior to use. This will reduce the background of the fluorescence assay.

Lipase (MAK564C)

Reconstitute with 685 µL of Triglyceride Assay Buffer. Mix well by pipetting, then aliquot and store at -20 °C. Keep on ice while in use. Use within 2 months of reconstitution.

Triglyceride Enzyme Mix (MAK564D)

Reconstitute with 220 µL of Ultrapure water (buffer addition will cause salt precipitates). Mix well by pipetting, then aliquot and store at -20 °C. Keep on ice while in use. Use within 2 months of reconstitution.

Triglyceride Standard (MAK564E)

Storage at 20 °C may cause the Triglyceride Standard to separate from the aqueous phase. Redissolve before use. Tightly close the cap and place the vial in boiling water for 1 minute (the solution will turn cloudy). Vortex for 30 seconds until the solution becomes clear. Repeat the heat and vortex step one more time. The Triglyceride Standard solution can then be used as supplied.

Procedure

All samples and standards should be run in technical duplicates or triplicates.

Triglyceride Standards for Colorimetric Detection

1. Dilute 60 µL of the 1 mM Triglyceride Standard with 240 µL of Triglyceride Assay Buffer to prepare a 0.2 mM standard solution.
2. Add 0, 2, 10, 20, 30, and 40 µL of the 0.2 mM standard solution into a 96 well plate, generating 0 (blank), 0.4, 2, 4, 6, and 8 nmole/well standards.
3. Add Triglyceride Assay Buffer to each well to bring the volume to 50 µL.

Triglyceride Standards for Fluorometric Detection

1. Prepare a 0.2 mM Triglyceride Standard as for the colorimetric assay.
2. Dilute 40 µL of the 0.2 mM Triglyceride Standard solution with 360 µL of the Triglyceride Assay Buffer to prepare a 0.02 mM Triglyceride Standard Solution.
3. Add 0, 10, 20, 30, 40, and 50 µL of the 0.02 mM standard solution into a 96 well plate, generating 0 (blank), 0.2, 0.4, 0.6, 0.8, and 1.0 nmol/well standards.
4. Add Triglyceride Assay Buffer to each well to bring the volume to 50 µL.

Detection sensitivity is 10–100 fold higher for a fluorometric assay.

Sample Preparation

Use ultrapure water for the preparation of samples.

Both the colorimetric and fluorometric assays require 50 µL of sample for each reaction (well). Samples may be assayed directly.

Serum samples containing 0.1–6 mM triglyceride can be assayed directly.

Milk should be diluted with 1% Tergitol assay buffer and placed in boiling water for 1 minute. Vortex for 30 seconds. Heat and vortex step should be repeated once.

Tissues (10 mg) or cells (~2 X 10^6) can be homogenized in 100 µL of ice-cold 1% Tergitol assay buffer for 10 minutes on ice. Centrifuge the samples at 12,000 × g for 5 minutes to remove insoluble

material. Collect the supernatant and dilute with buffer before assay.

Add 2–50 µL of samples into duplicate wells of a 96 well plate. Bring samples to a final volume of 50 µL with Triglyceride Assay Buffer.

Note: For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the range of the standard curve.

A sample background control should be performed by replacing 2 µL of Lipase with 2 µL of Triglyceride Assay Buffer. The background should be subtracted from all readings.

Endogenous compounds may interfere with the assay. To ensure accurate determination of triglycerides in the test samples, it is recommended to spike samples with a known amount of Triglyceride Standard (example 4 nmole for colorimetric and 0.4 nmole for fluorometric).

Lipase Treatment

Add 2 µL of Lipase to each sample and standard reaction (well). Mix well and incubate for 20 minutes at room temperature to convert triglyceride to glycerol and fatty acid.

Note: If samples contain glycerol, include a sample background control for each sample by omitting the Lipase to determine glycerol background only.

Assay Reaction

1. Set up the Master Reaction Mixes according to Table 1. 50µL of the appropriate Reaction Mix is required for each reaction (well)

Table 1.
Master Reaction Mix Preparation

Reagent	Samples and Standards
Triglyceride Assay Buffer	46 µL
Triglyceride Probe	2 µL
Triglyceride Enzyme Mix	2 µL

2. Add 50 µL of the Master Reaction Mix to each of sample, standard, and background control well containing the triglyceride standard. Mix well using a horizontal shaker or by pipetting.

3. Incubate the plate for 30–60 minutes for the colorimetric assay and 90-120 minutes for the fluorometric assay at room temperature. Protect the plate from light during the incubation.

4. For the colorimetric assay, measure the absorbance at 570 nm (A570). For the fluorometric assay, measure fluorescence intensity (λ_{ex} = 535/λ_{em} = 587 nm).

The reaction is stable for at least 2 hours.

Results

Calculations

The background for the assay is the value obtained for the 0 (blank) Triglyceride Standard. Correct for the background by subtracting the blank standard value from all readings. Background values can be significant and must be subtracted from all readings. Subtract the Sample Background Control value from the sample readings.

Use the values obtained from the appropriate Triglyceride standards to plot a standard curve. The amount of Triglyceride present in the samples may be determined from the standard curve.

Note: A new standard curve must be set up each time the assay is run.

For spiked samples, correct for interference by using the following equation (for colorimetric and fluorometric):

$$\left\{\frac{(\lambda/OD_{\text{Sample corrected}})}{(\lambda/OD_{\text{Spiked corrected}})-(\lambda/OD_{\text{Sample corrected}})}\right\} \times \text{TGSpike(nmol)}$$

Concentration of Triglyceride

Sa/Sv = C

Sa = Quantity of Triglycerides in the unknown sample (nmole) from standard curve.

Sv = Sample volume (µL) added into the wells.

C = Concentration of Triglyceride in sample.

Triglyceride-triolein molecular weight: 885.43 g/mole.

Sample Calculation

Amount of Triglyceride (Sa) = 5.84 nmole (from standard curve).

Sample volume (Sv) = 50.0 µL.

Concentration of Triglyceride in sample:

5.84 nmole/50.0 µL = 0.117 nmole/µL.

0.117 nmole/µL × 885.43 ng/nmole = 104 ng/µL.

Figure 1:
An exemplary colorimetric standard curve.

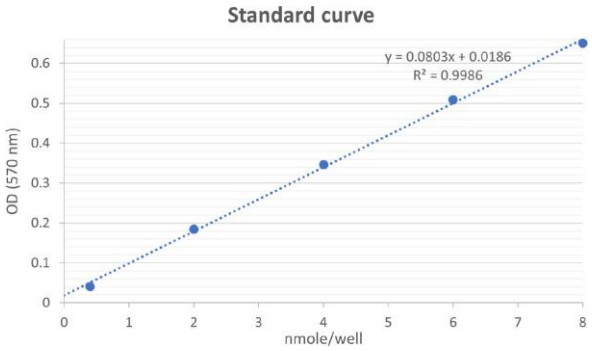


Figure 2:
An exemplary fluorometric standard curve.

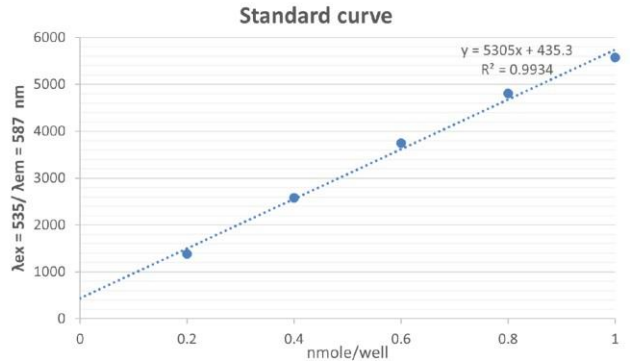
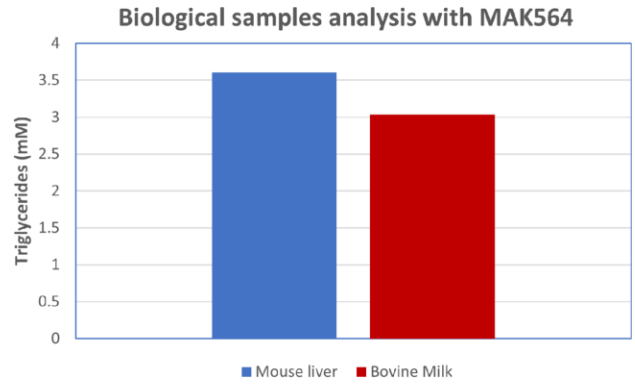


Figure 3:
Example of biological sample analysis.



Mouse liver – 10 mg tissue analysis.

Bovine milk - Analysis of 3% fat milk.

References

Talayero, B.G, and Sacks, F.M., The role of triglycerides in atherosclerosis. *Current Cardiology Reports,* **13(6)**, 544-52 (2011)

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Ice Cold Assay Buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates. For colorimetric assays, use clear plates.
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
	Improperly thawed components	Thaw all components completely and mix gently before use
Lower/higher readings in samples and standards	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
Non-linear standard curve	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

Technical Service

Visit the tech service page on our web site at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.

mak564pis Rev 09/24