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Product Information

Triglyceride Quantification Kit

Catalog Number **MAK266**
Storage Temperature –20 °C

TECHNICAL BULLETIN

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 are 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.

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 2 pmole–10 nmole (2–10,000 μM range) of triglyceride in various samples. The kit also detects monoglycerides and diglycerides.

This kit is suitable for use with serum, plasma, and other biological fluids, and tissue and cell culture samples.

Components

The kit is sufficient for 100 assays in 96 well plates.

Component	Amount
Triglyceride Assay Buffer Catalog Number MAK266A	25 mL
Triglyceride Probe, in DMSO Catalog Number MAK266B	0.2 mL
Lipase Catalog Number MAK266C	1 vl
Triglyceride Enzyme Mix Catalog Number MAK266D	1 vl
Triglyceride Standard, (1 mM) Catalog Number MAK266E	0.3 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use white plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader
- Nonidet™ P 40 Substitute (Catalog Number 74385)

Precautions and Disclaimer

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

Preparation Instructions

Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Triglyceride Assay Buffer – Allow buffer to come to room temperature before use.

Triglyceride Probe – 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.

Triglyceride Enzyme Mix - Reconstitute with 220 μL of Triglyceride Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Use within 2 months of reconstitution.

Lipase - Reconstitute in 220 μL of Triglyceride Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Use within 2 months of reconstitution.

Triglyceride Standard - 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 a hot water bath (80–100 °C) 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.

Storage/Stability
The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Triglyceride Standards for Colorimetric Detection
Dilute 40 μL of the 1 mM Triglyceride Standard with 160 μL of Triglyceride Assay Buffer to prepare a 0.2 mM standard solution. Add 0, 10, 20, 30, 40, and 50 μL of the 0.2 mM standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Triglyceride Assay Buffer to each well to bring the volume to 50 μL.

Triglyceride Standards for Fluorometric Detection
Prepare a 0.2 mM Triglyceride Standard as for the colorimetric assay. Dilute 20 μL of the 0.2 mM Triglyceride Standard solution with 180 μL of the Triglyceride Assay Buffer to prepare a 0.02 mM Triglyceride Standard Solution. Detection sensitivity is 10–100 fold higher for a fluorometric assay. 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. Add Triglyceride Assay Buffer to each well to bring the volume to 50 μL.

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.

Tissue (100 mg), cells (1×10^7), or other non-aqueous samples can be homogenized in 1 mL solution of 5% Nonidet P 40 Substitute (made by dissolving 0.25 g of Catalog Number 74385 in 5 mL water, or equivalent). Slowly heat the samples to 80–100 °C in a water bath for 2–5 minutes or until the Nonidet P 40 becomes cloudy, then cool to room temperature. Repeat the heating one more time to solubilize all triglycerides. Centrifuge for 2 minutes at top speed to remove insoluble material. Dilute 10-fold with water 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.

Notes: 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).

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 Mix according to the scheme in Table 1. 50 μL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Samples, Standards, and Background Controls
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 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 at room temperature. Protect the plate from light during the incubation.

4. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity (λ_{ex} = 535/λ_{em} = 590 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:

$$\frac{OD_{sample\,(corrected)}}{(OD_{sample+TG\,Std\,(corrected)}) - (OD_{sample\,(corrected)})} * \text{TG spike (nmol)}$$

Concentration of Triglyceride

$$S_a/S_v = C$$

S_a = Amount of Triglyceride in the unknown sample (nmole) from standard curve
S_v = Sample volume (μL) added into the wells
C = Concentration of Triglyceride in sample

Triglyceride (triolein) molecular weight: 885.4 g/mole

Sample Calculation

Amount of Triglyceride(S_a) = 5.84 nmole (from standard curve)

Sample volume (S_v) = 50.0 μL

Concentration of Triglyceride in sample

$$5.84 \text{ nmole}/50.0\ \mu L = 0.117 \text{ nmole}/\mu L$$

$$0.117 \text{ nmole}/\mu L \times 885.4 \text{ ng/nmole} = 104 \text{ ng}/\mu L$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay Not Working	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 filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms. 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 samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	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 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
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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