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Product Information

High Sensitivity Triglyceride (TG) Assay Kit

Catalog Number **MAK264**
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 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 High Sensitivity Triglyceride Assay Kit is suitable for measuring triglyceride levels in samples that contain reducing substances that may interfere with oxidase-based assays. In this assay, TG is hydrolyzed with lipase to glycerol and fatty acids. The glycerol is used in a coupled enzyme reaction, which results in a fluorometric product (λ_{ex} = 535 nm/λ_{em} = 587 nm), proportional to the amount of triglycerides present. This high-throughput, adaptable assay kit is simple and sensitive with the capability to detect less than 0.4 µM of triglycerides in a variety of samples.

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

Components

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

Triglyceride Assay Buffer 25 mL
Catalog Number MAK264A

High Sensitivity Probe, in DMSO 0.4 mL
Catalog Number MAK264B

Lipase 1 vl
Catalog Number MAK264C

Triglyceride Enzyme Mix 1 vl
Catalog Number MAK264D

Triglyceride Developer Mix 1 vl
Catalog Number MAK264E

Triglyceride Standard, (1 mM) 0.3 mL
Catalog Number MAK264F

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate. It is recommended to use black plates with clear bottoms for fluorescence assays.
- Fluorescence multiwell plate reader

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. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

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

High Sensitivity Probe – Warm to room temperature to thaw the solution prior to use. Store at –20 °C. Upon thawing, the probe is ready to use as supplied.

Lipase and Triglyceride Enzyme Mix – Reconstitute with 220 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 Developer – Reconstitute in 220 µL of water. 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 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.

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. Use ultrapure water for the preparation of samples.

Triglyceride Standards for Fluorometric Detection
Dilute 20 µL of the 1 mM Triglyceride Standard with 980 µL of water to prepare a 20 µM (20 pmole/µL) standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 20 µM standard solution into a 96 well plate, generating 0 (blank), 40, 80, 120, 160, and 200 pmole/well standards. Add Triglyceride Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Fluorometric assays require 50 µL of sample for each reaction (well).

Serum samples can be assayed directly.

Tissue (10 mg) or cells (1×10^6) can be homogenized in 100 µL of ice-cold Triglyceride Assay Buffer for 10 minutes on ice. Centrifuge the samples at 12,000 × *g* for 5 minutes to remove insoluble material. Collect the supernatant.

Saliva should be centrifuged at 5,000 × *g* for 2 minutes. Collect the supernatant

Add 1–50 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.

NADH or NADPH present in the sample can generate background. To control background, include a sample control for each sample by omitting the Triglyceride Enzyme Mix in the Reaction Mix. The Sample Blank readings can then be subtracted from the sample readings.

Lipase Treatment
Add 2 µL of Lipase to each of the sample wells and standard wells. Mix and incubate at 37 °C for 20 minutes.

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 and Standards	Sample Blank
Triglyceride Assay Buffer	45 µL	47 µL
High Sensitivity Probe	1 µL	1 µL
Triglyceride Enzyme Mix	2 µL	–
Triglyceride Developer	2 µL	2 µL

2. Add 50 µL of the Master Reaction Mix to each sample and standard control well. If using a sample control, add 50 µL of Sample Blank Mix to sample control wells. Mix well using a horizontal shaker or by pipetting.

3. Incubate the plate for 30 minutes at 37 °C. Protect the plate from light during the incubation.

4. Measure fluorescence intensity (λ_{ex} = 535/ λ_{em} = 587 nm).

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. Use the values obtained from the appropriate Triglyceride Standards to plot a standard curve.

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

Subtract the Sample Blank value from the sample readings to obtain the corrected fluorescence measurement. Using the corrected fluorescence measurement, determine the amount of Triglyceride present in the sample from the standard curve.

Concentration of Triglyceride

$$S_a/S_v = C$$

S_a = Amount of Triglycerides in the unknown sample (pmole) from standard curve
S_v = 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 (S_a) = 162.5 pmole (from standard curve)
Sample volume (S_v) = 25 µL

Concentration of Triglyceride in sample:

$$162.5 \text{ pmole}/25 \text{ µL} = 6.5 \text{ pmole}/\text{µL}$$

$$6.5 \text{ pmole}/\text{µL} \times 885.43 \text{ pg/pmole} = 5755 \text{ pg}/\text{µL}$$

References

1. 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	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.
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 aged kit or improperly stored reagents	Check the receipt 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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